StatSTEM tutorial

Quantitative (S)TEM by using StatSTEM

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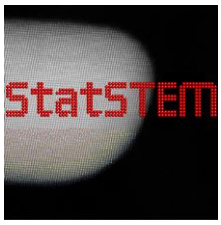

1. Introduction

This lab session is intended to introduce the StatSTEM software and its capabilities in the field of quantitative (scanning) transmission electron microscopy ((S)TEM).

The StatSTEM software is an open source code written in MATLAB. The code and a manual can be found on the StatSTEM website: https://github.com/quantitativeTEM/StatSTEM under the releases button. In this lab session, a compiled version will be used. More details on the algorithm can be found in: Ultramicroscopy 171 (2016) 104-116.

In the instructions that follow, **bold letters** refer to a button or menu in the program while *italic letters* refer to a panel within the program. In the top left corner different TOOLS are available which will be active for the shown image:

Please ask for help if the instructions are not clear enough!

Launch StatSTEM by double clicking on its icon on the desktop.

2. A general overview on StatSTEM its functionalities

2.1 The colormap

In this first part, we will go briefly through the different functionalities that StatSTEM has to offer.

Click on **Load** and open the image "ExamplePtIr.txt". StatSTEM will ask for the pixel size, which is 0.1211Å.

A grayscale image of a normalised Pt/Ir particle is loaded. The grayscale of the image can be modified by adding a colorbar to the image:

By right-clicking on the inserted colorbar, the colormap of the image can be changed. Activate for example the jet colormap: **Standard Colormaps→jet**

Since most electron microscopy images are shown in grayscale, this colormap is set as the standard colormap in StatSTEM. Change back to the grayscale colormap: **Standard Colormaps→gray**

Sometimes the most interesting features of an image are only present within a specific range of pixel values. Edit the range of pixel values by right-clicking on the colorbar and change the range: **Open Colormap Editor**

Restore the colormap range by right-clicking on the colorbar: **Reset Range Colors**

When the range of colors is optimised, you can further highlight different features in the image by right-clicking on the colorbar: **Interactive Colormap Shift**
By using this function you can drag a color in the colorbar to a different value.

Restore the colormap by right-clicking on the colorbar: **Standard Colormaps→gray**

Can you think of any features in an image that can be highlighted by using the colorbar?

2.2 Starting values

Continue using the image "ExamplePtIr.txt".

Before modelling an image as a superposition of Gaussian peaks, initial column positions should be given. For this purpose, StatSTEM offers a peak finding routine and the possibility to give manually starting values. Furthermore, starting positions of the columns can be loaded from an external file.

We start by using the peak finding routine: **Peak Finder Routine→Tune parameters**

The peak finder routine searches for local maxima in your image. Since noise is always present in the experimental images, different filters can be used. The parameters of these filters can be tuned in the new window that has been opened. Standard, a Gaussian filter is used with a width of 2 pixels. Also a threshold value can be given. The pixel values should have higher values to be found as an atomic column position.

Add a filter and modify the parameters of the filters and the threshold value to see how it affects the image.

Restore the parameters to the initial values by: **Reset values**
A threshold value of 0 and a Gaussian filter with a width of 2 pixels will be used.

Test the peak finder routine: **Test peak finder**

Can you explain why it finds peaks everywhere in the image, even where there are no atomic columns?

Set the threshold value to 0.02 and test the peak finder routine again: **Test peak finder**

After suitable parameters and filters have been selected, the parameters can be exported to StatSTEM: **Store and close**

In StatSTEM, execute the peak finder routine: **Peak Finder Routine →Execute routine**

As can be seen, the peak finder routine did not find initial values for all column positions. Manually add positions for the columns that you can visually see: **Add/Remove Peaks→ Add**

The last option is to load coordinates from an external file. Load the column positions from the file "CoordinatesPtIr.txt": **Import peak locations → Load file**

Are the loaded column positions similar as compared to your initial values? If not, can you think of a reason that explains the difference?

2.3 Modelling the image

After initial starting values are given for the column positions, the image can be modelled as a superposition of Gaussian peaks. Go to the next tab *Fit Model*.

In this panel different options are available:

- Fit a constant background
- Model the column intensities by Gaussian peaks having all the same width, all a different width, or a fixed given width
- Test for convergence
- Parallel computing

For the moment use the standard values: fit a constant background, use the same width for all the Gaussian peaks and don't use test conditions. Start the fitting procedure: **Run fitting routine**

The fitting procedure may take a while. Once it is finished, the fitted model will be shown. Compare the fitted model to the original image by selecting the shown image in the right top corner: **Select image → Observation** and **Select image → Model**

Show the model: **Select image → Model** and make both the input and fitted coordinates visible: **Image Options → Input coordinates**

Is there much difference between the initial starting positions and the fitted positions?
A large difference between the input and fitted positions indicates that the initial values were most likely not accurate enough and might need an update.

2.4 Atom counting

After the image is modelled, structure parameters can be extracted. One of the structure parameters has already been discussed: the coordinates of the atomic columns. Another structure parameter is the so-called scattering cross-section (SCS). The SCS is the total scattered intensity by an atomic column and scales in HAADF STEM imaging with both the thickness and the chemical composition of the atomic column. The SCS can be obtained by integrating the total intensity scattered by an atomic column, which is done in StatSTEM by using the volumes of the Gaussian peaks. Here, both the height and width of each Gaussian peak is used to calculate the volume. In StatSTEM, the SCSs of all the different columns can be seen in a histogram: **Select Image → Histogram SCS**

How should the histogram of the SCSs look in the case that no image noise and distortions are present?

Despite the noise that is present in the measured values of the SCSs, we still want to perform atom counting. Visually, it is impossible to identify the number of atoms that should be associated with each measured value of the SCS from the histogram. Therefore a statistics- and a simulation-based method will be discussed. Go to the next panel: *Analysis*.

2.4.1 Atom counting – Statistics-based method

The first method to perform atom counting is by using a statistics-based method in which the SCSs are modelled by a Gaussian mixture model. It is, however, unknown how thick the particle is and how many Gaussian components are present in the histogram. In order to decide how many Gaussian components are present in the histogram, the ICL criterion will be used. This criterion will show a local minimum at the correct number of Gaussian components in the histogram.

In order to use the ICL criterion to count the number of atoms, the SCSs in StatSTEM will be modelled by a Gaussian mixture model for an increasing number of Gaussian components. Open the options: **Atom counting – Statistical → Show options**

In the standard settings, the maximum number of Gaussian components is 50. For the moment this is fine, as we don't expect that the column thickness of the particle is larger than 20 atoms. Therefore, you can also change this number to reduce the computation time: **Atom counting – Statistical → Pre-analysis options → Max. components → 20**

How can we determine an appropriate value for the maximum number of Gaussian components?

Start the fitting of the Gaussian mixture model and the ICL procedure: **Atom counting – Statistical → Run ICL**

When the procedure is finished, the user is asked to click on the local minimum. Select a minimum which you think is suitable. After selecting the minimum the experimental image is shown together with the counting results.

Open the ICL image: **Select image → ICL**
Select another minimum and see how the counting results change: **Atom counting – statistical → Post-analysis options → Select new ICL minimum**

Open the histogram: **Select image → Histogram SCS**
In this image, the Gaussian mixture model is shown together with the individual components. The colors of the individual components are directly related to the colors of the atom counts in the experimental image.

Open the graph with the SCSs as a function of thickness: **Select image → SCS vs. thickness**
Here, the locations of the different Gaussian mixture model components are shown. Often the component number is directly related to the number of atoms. Select the local minimum 16 in the

ICL, if not yet selected: **Post-analysis options → Select new ICL minimum** and go back to the graph **Select image → SCS vs. thickness**

From this graph it can be seen that the SCSs increase monotonically with thickness. It should be noted that this increase is not a linear increase due to the channeling of the electrons along the columns.

2.4.2 Atom Counting – Simulation-based method

Another method to count the number of atoms is by comparing with image simulations. By using, for example, the MULTEM software simulated images can be acquired as a function of specimen thickness. The SCSs of the columns in the simulated image as a function of thickness serve as a library for atom counting. In this method, the experimentally measured SCSs are directly compared to the library values.

Load the library "LibraryPtIr.txt": **Atom counting – Simulation → Load library values**

In the graph showing the SCSs as a function of thickness, **Select image → SCS vs. thickness**, the loaded library is shown together with the experimental locations of the Gaussian components in the mixture model. Do the library values match with the experimental values?

Select another minimum in the ICL and find a match between the library and the experimental values: **Post-analysis options → Select new ICL minimum**

What minimum did you find? What is the percentage of atomic columns counted with single atom sensitivity (see the message panel)?

The library values can also be used in StatSTEM to perform atom counting: **Atom counting – Simulation → Match with simulations**

In the experimental image, compare the counting results from the simulation-based method and the statistics-based method: **Image Options → Atom Counts** and **Image Options → Lib Counts**

Are there any differences? How can you explain the differences?

2.5 Strain mapping

Another feature of StatSTEM is atomically resolved strain mapping. Go to the analysis panel: *Analysis*.

For strain mapping, a necessary input to StatSTEM is the projected unit cell: **Strain mapping → Projected unit cell**

In this new window one can design its own projected unit cell. For Pt in the 110 direction there is already a database file "DatabaseStrain/Pt110.txt". Load this file: **Load file**

Export the file to StatSTEM: **Export to StatSTEM**

Open the strain mapping options: **Strain mapping → Show options**

Deselect the atom counting results: **Image Options → Atom Counts**

First a reference coordinate should be given, in which no strain of a minimum amount of strain is expected. Standard the most central coordinate is chosen: **Strain mapping → Reference coordinate → Most central → Show**
If this coordinate is not suitable, another coordinate can be chosen manually: **Strain mapping → Reference coordinate → User defined → Select** and select a coordinate in the image.

In this particle, the most central atomic column experiences most likely less strain as compared to the others: **Strain mapping → Reference coordinate → Most central → Show**

Next, the direction of the lattice parameters should be found. This procedure requires an accurate value for the pixel size of the image! An automatic procedure is available to find the a-direction of the unit cell in the image: **Strain mapping → Direction a lattice → Automatic → Show**

Otherwise, the a-direction can be given manually. In the procedure of finding the a-direction, the given lattice parameter can be updated by a fitting procedure. Standard, a region of 3 unit cells around the selected reference coordinate is used to update the value of the lattice parameter a by a fitting procedure. If this is unwanted, this option can be deselected. For now, leave this option turned on.

After selecting a reference coordinate and finding the a-direction, a displacement map can be made: **Strain mapping → Make displacement map**

A warning will be given that not all points are found. This is caused by the loose atoms that are not packed to the Pt/Ir particle. Accept the warning and manually select a region around the particle which neglects the loose atoms: **Strain mapping → Show options** next select **Strain mapping → General options → Select columns in image**

Create again a displacement map: **Strain mapping → Make displacement map**

In this displacement map, the fitted coordinates are compared to the expected ideal lattice positions when no strain is present in the sample.

The red arrows indicate the displacement of the atomic column positions with respect to expected atomic columns of the ideal lattice. The displacement of the atomic columns at the edge of the particle is largest, why?

After the displacement map is created, a strain map can be created by using a first derivative of the displacement vectors: **Strain mapping → Make strain map**

Standard, the ε_{xx} map is shown. Look also at the other strain maps: **Image Options → ε_{xy}** , **Image Options → ε_{yy}** and **Image Options → ω_{xy}**. In which atomic columns is most strain present? Is this in agreement with the displacement map?

2.6 Store and close image

When you are finished analyzing an image, you can store the results in a MATLAB file: **Save**
After saving the results, close the image in StatSTEM by right-clicking on the image tab: *ExamplePtIr* → **Close image**

Afterwards you can reopen the saved file in StatSTEM or MATLAB to modify or extract some of the results

3. Atom counting in experimental images

In this part of the tutorial, you will try to fit a Gaussian model and perform atom counting on a Pb particle. Load the image "ExamplePb.mat": **Load**

One of the difficult tasks in fitting a Gaussian model in a correct manner is finding appropriate starting coordinates. Therefore, we will first focus on this part.

Go to the tab: *Preparation*

Use the peak finder routine to find an initial guess of starting values of the column positions. Tune the parameters and filters of the routine: **Peak Finder Routine → Tune parameters**

You will see that the routine may also find column positions in the top left and top right part of the image, this is not a problem. Here intensities are observed which belong to other particles. Therefore, you may assign some starting values to model also the column positions of these particles.

After you have found good settings, export them to StatSTEM: **Store and close**
and run the routine in StatSTEM: **Execute routine**

Most likely the peak finder routine will not find all column positions or finds too many columns. Therefore, manually add and remove column positions: **Add/Remove Peak → Add** and **Add/Remove Peak → Remove**

Try to change the colormap of your image by using the colorbar introduced in part 1.1 of this tutorial to highlight the image intensities belonging to the particle.

After you have found starting values, go to the next tab: *Fit Model*. At this moment, you still may have assigned too many column positions or too little. Therefore, the 'test for convergence' option in StatSTEM may be used. Visualize the options: **Show options** and enable the test option: **Test for convergence → Use test conditions**
In this case, the maximum number of iterations is limited to 4 for finding the column positions.

Before starting the fitting procedure, make sure that you will fit a constant background to the image and fit all column positions as Gaussian peaks having the same width. Run the fitting procedure: **Run fitting routine**

After the fitting procedure is finished, a Gaussian model of the image will be shown. Make sure that the fitted and input coordinates agree quite well and check if your starting values are all fine. If only a few atomic column positions are fitted incorrectly, you may want to make the fitted column coordinates the new input coordinates by using the button: **Test for convergence → Export fitted coordinates**

Continue this process of adding and removing column positions and fitting the model in the test case scenario until you have found robust starting values. Then deselect the test option: **Test for convergence → Use test conditions** and fit the Gaussian model to the image: **Run fitting routine**

If you do not use appropriate initial values, the fitting routine will not be able to converge and the fitting will take very long. If the fit takes longer than 5 minutes and if the progress bar remains between 70-74%, you are probably not using appropriate initial positions. To interrupt the fitting procedure: **Abort fitting routine**
Repeat the procedure where you test for convergence.

If you have any doubt about the initial values, ask any of the assistants!

Once the image is modelled, we can continue with the atom counting procedure. Most likely you have modelled also some column positions of the particles at the edge of the image. These column positions should not be included in the atom counting procedure. Therefore, in the *Analysis* panel, show the options of the statistical method for atom counting: **Atom counting – Statistical → Show options**. Select only the atomic column belonging to the central particle: **Atom counting – Statistical → Pre-analysis options → Select columns in image**

Since the particle is small, we do not expect that the atomic columns contain more than 30 atoms: **Atom counting – Statistical → Pre-analysis options → Max. components: → 30**

Start the fitting of the Gaussian mixture model with the ICL criterion: **Atom counting – Statistical → Run ICL** and select a minimum.

If you show the histogram, **Select Image → Histogram SCS**, you may see the different components of the Gaussian mixture model. Open the graph showing the SCSs as a function of thickness, **Select Image → SCS vs. Thickness**, and check if the increase of the SCSs with thickness is monotonic.

Open a second image "Example2.mat": **Load**

Compare your results with the loaded results and check if there are any differences. If so, can you explain the differences?

Save your results, **Save**, and close all opened images by right clicking on the image panel names, *ExamplePb* → **Close image** and *Example2* → **Close image**

4. Different column types

Many nanoparticles are composed of different atom types. Therefore, the composition of the columns may change. A different composition of a column may also result in a different width of the column intensity. Therefore, it is better to fit the Gaussian model for images of heterogeneous materials by assuming a different width for each column type. In this section, we will discuss how you can do this in StatSTEM.

First load the image "ExamplePbCsBr3.mat": **Load**

Go to the panel *Preparation.*

Tune the peak finder routine, **Peak Finder Routine → Tune parameters**, by changing the threshold value to "8500". Export the parameters to StatSTEM: **Store and close**

Execute the peak finder routine: **Peak Finder Routine → Execute routine**

At this moment, only one column type is present in StatSTEM named "1". Add another type: **Add/Remove Peak → Type: → 1 → Add**
Give the new column type the name: "Cs".
Rename column type 1: **Add/Remove Peak → Type: → Cs → Names** and modify the new name to: "Pb".

Standard columns of the first type, Pb, are colored green. Columns of the 2nd type are colored red.

Change the column type to Cs: **Add/Remove Peak → Type: → Pb → Cs**

In the image, the brightest columns are the Pb columns while the columns with intermediate brightness are the Cs columns. Change the type of these Cs columns by using: **Add/Remove Peak → Change type to:**
With this function you can select a region around the Cs columns.

In a future release of StatSTEM, a more efficient function will be implemented to select the different atom types. It is also always possible to load a user-defined input file which defines the different atom types.

After this, add manually starting column positions to the image and/or correct for missing or double starting coordinates: **Add/Remove Peak → Add** and **Add/Remove Peak → Remove**
Make sure that for adding new column positions, the correct type is selected: **Add/Remove Peak → Type: → Pb/Cs!**

Use the tricks discussed in section 1.1 and section 2 to find all the column positions. Also make use of the 'test for convergence' option for fitting the Gaussian model and checking the correctness of your starting coordinates. For fitting the Gaussian model, use the standard "same" width approach. In this approach, only columns having the same type will be modelled as Gaussian peaks having the same width.
After you have found appropriate starting values, you can fit the Gaussian model from the panel *Fit Model*, without using test conditions.

After fitting the Gaussian model, show the histogram: **Select Image → Histogram SCS**

You will notice that the histogram shows two peaks, why?

As this image is constant in thickness, we cannot perform atom counting using the statistics-based method. For the statistics-based atom counting method, a varying thickness is needed to make the SCSs of the different column thicknesses present in the histogram. What method can still be used to perform atom counting?

For this fitted model, we will only apply strain mapping. Go to the panel *Analysis.*

Show the options of the strain mapping: **Strain mapping → Show options**

Load the projected unit cell "CsPbBr3_100.txt": **Strain mapping → Projected unit cell → Load**
Export it to StatSTEM: **Export to StatSTEM**

Select a column in the centre of the image as a reference coordinate: **Strain mapping → Reference coordinate → User defined**
Standard the first column type, here Pb, is used for selecting a reference coordinate. This can be changed if wanted: **Strain mapping → Reference coordinate → Type**
For now, we will use the Pb column type.

Manually select the direction of the a-lattice with respect to the reference coordinate, which can be either horizontal or vertical in this image: **Strain mapping → Direction a lattice → User defined → Select**

Now, make the displacement map: **Make displacement map**
After a displacement map is made, create the strain maps: **Strain mapping → Make strain map**

Do you see parts in the image that are heavily strained? The particle that you have studied is NOT a bulk material, but a beam-sensitive nanoparticle. Can this explain your observation?

The figures that are created in StatSTEM can be saved by using the function: **Export figure → Export**
This opens a new window, where you can save the image, for example by **File → Save As…**

Load the file "Example3.mat": **Load**. Compare whether your results match with the example file.

After you are sure that your results match with the example file, you may save your results, **Save**, and close StatSTEM.