Multireference alignment
Use of plugins

Multireference and plugins

You have probably noticed the [nref] boxes foreseen in project_manager to setup multireference projects. Also, overall in *Dynamo* you have also seen edit fields allowing to specify a “ref” or “reference” number.

These slides show how to setup a project that includes several references, (or “reference channels”, as called in the *Dynamo* documentation).

There are two basic guidelines

1 – Each multireference channel needs its own seed files for
* initial table
* initial template
* initial fourier mask

This means that the syntax will be slightly different, as you need to pass multiple files.
This is resolved
a) by passing the name of a folder, and using a convention to name the individual files, or
b) by passing a .sel file, i.e., a text file that contains the names of the actual files

2- Each reference channel is an independent alignment process.
To create a real multireference experiment, the different channels need to interact with each other. There are many, many ways you might imagine such an interaction, and this is a natural battlefield for plugins.

This tutorial

In this tutorial we show how to use the *Dynamo* tutorial tool to create a correctly formatted multireference project on a provided data set, and how to connect it to a provided plugin..

Creating the tutorial data set

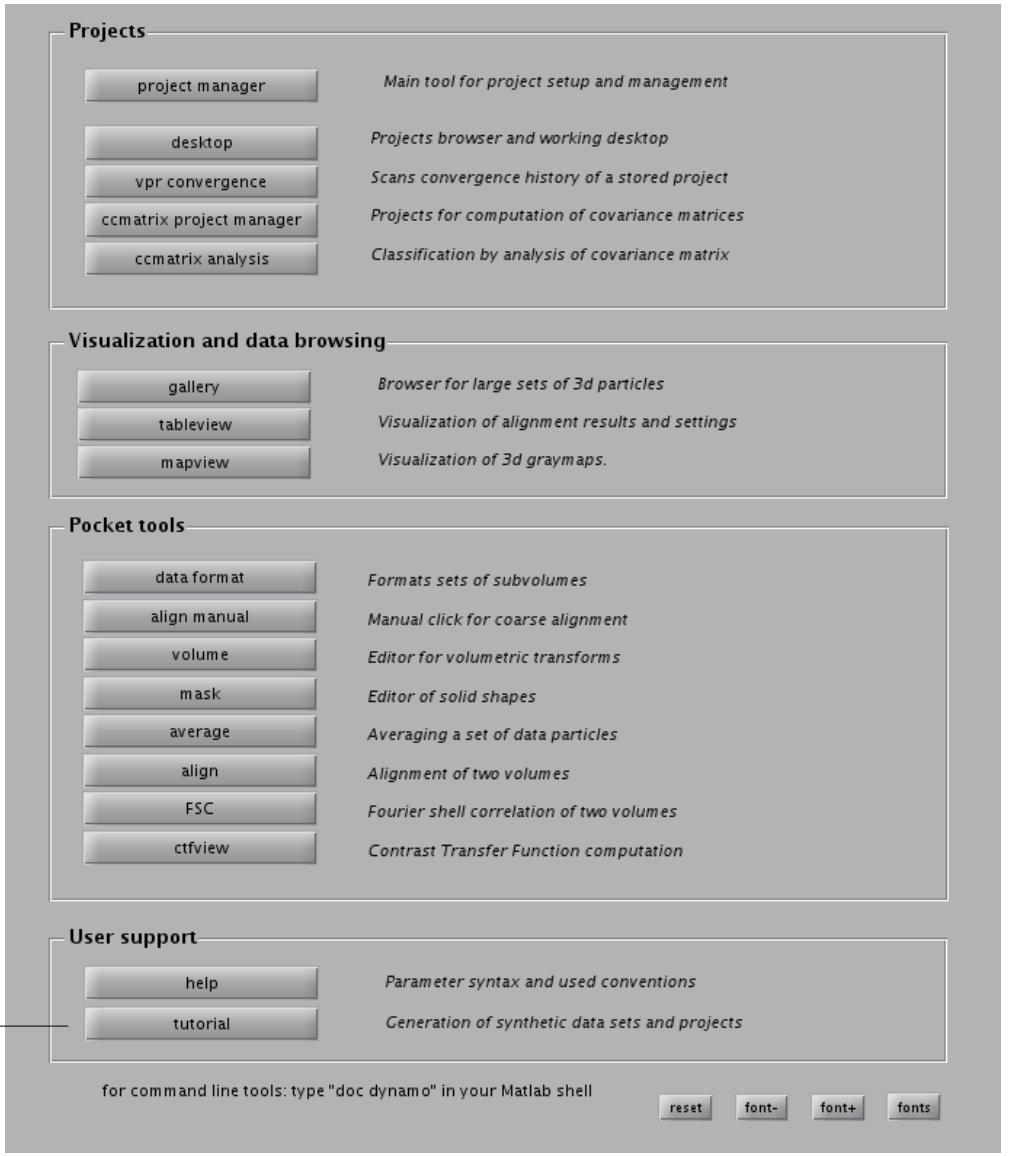
open the tutorial interface
by opening first the *Dynamo* tools:

Matlab (all platforms)
```
>> dynamo
```

Standalone Linux/MacOS
```
$ dynamo &
```

Standalone Windows
```
> dynamo.exe
```

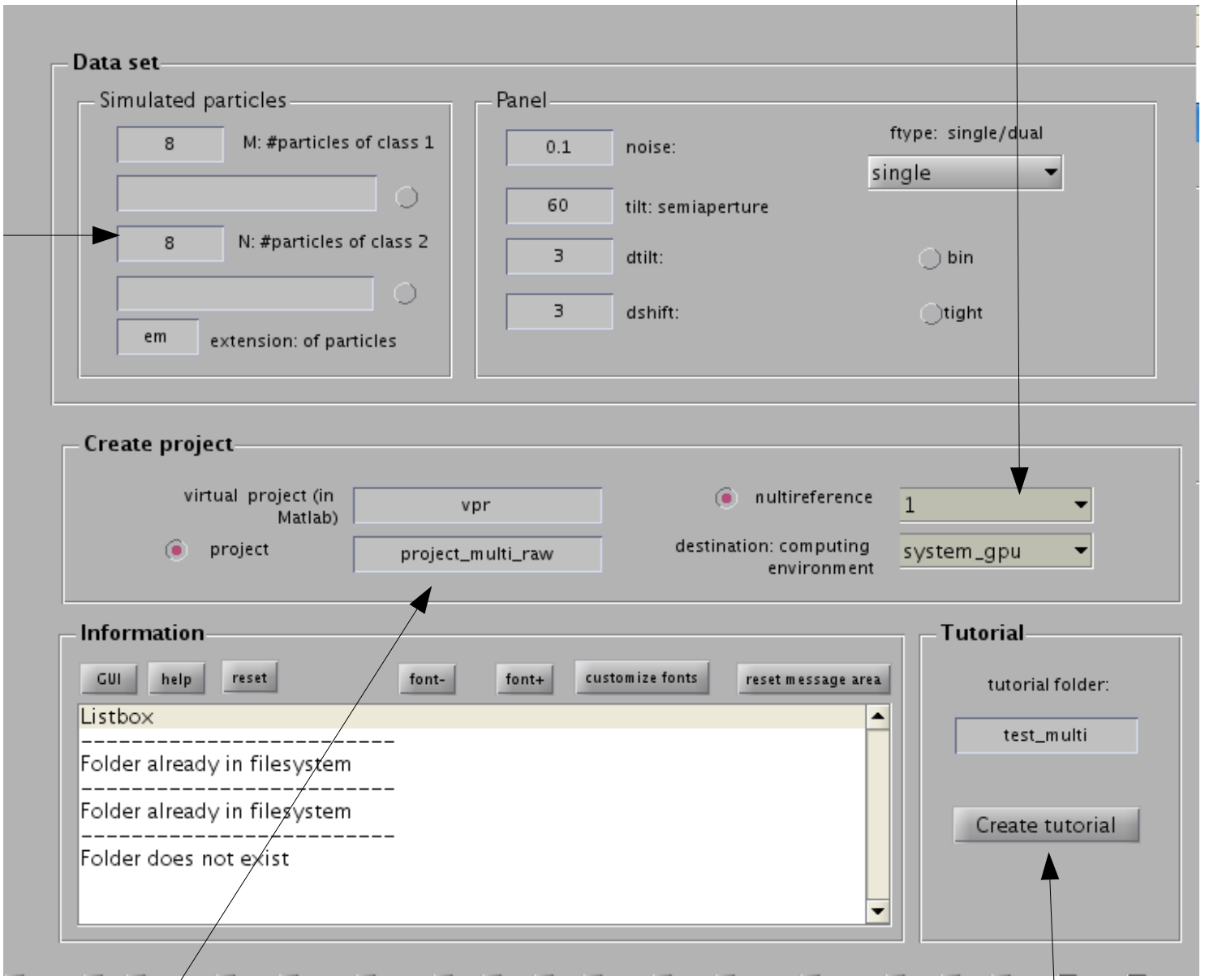
initiate a tutorial



We introduce some changes on the standard settings:

use the multireference option (of type 1 or 2 indistintly)

use 8 particles of the “second” class



put a recognizable name for this project

... And create the tutorial

We are producing a similar data set to the one we created to get familiar with the classification tools of *Dynamo*

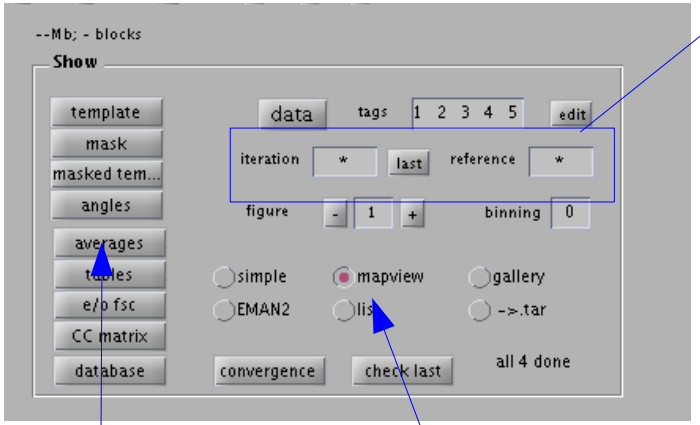
The data set will comprise 8 randomly oriented copies of a thermosome model and another 8 copies of the a slightly shrunken version of this model.

In the classification tutorial we used PCA analysis to separate the two classes.

Now, we will use a “classical” multireference approach implemented in a plugin to classify the particles by aligning them against different templates and letting them recognize the template that yields the best score.

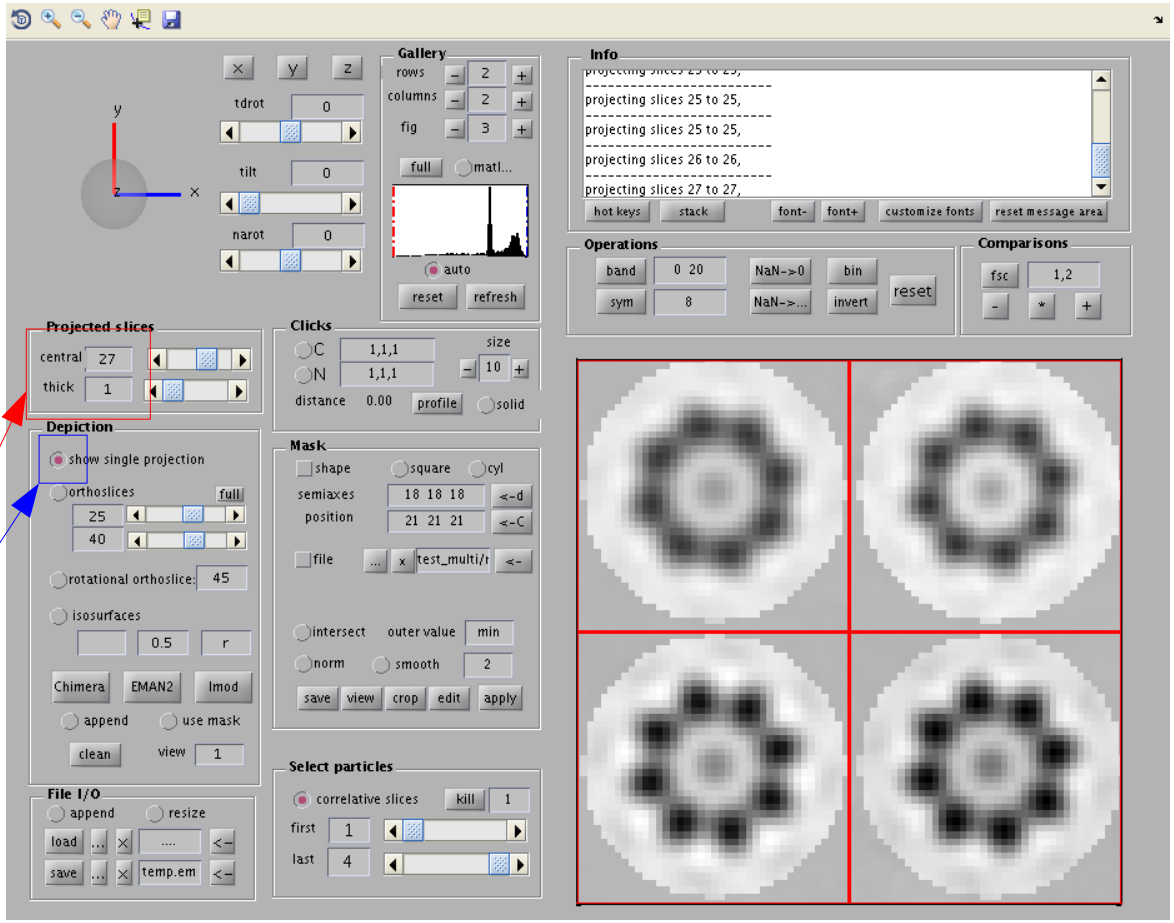
Take a look on the results:

1) select all (*) references and all (iterations)



2) select `mapview` as viewer

3) press on [averages]



inside `mapview` use these viewing settings

now, each row is the result of one iteration
each column is the result for one reference

The result s do not look very impressive: the averages provided by both “references channels” look pretty much the same.

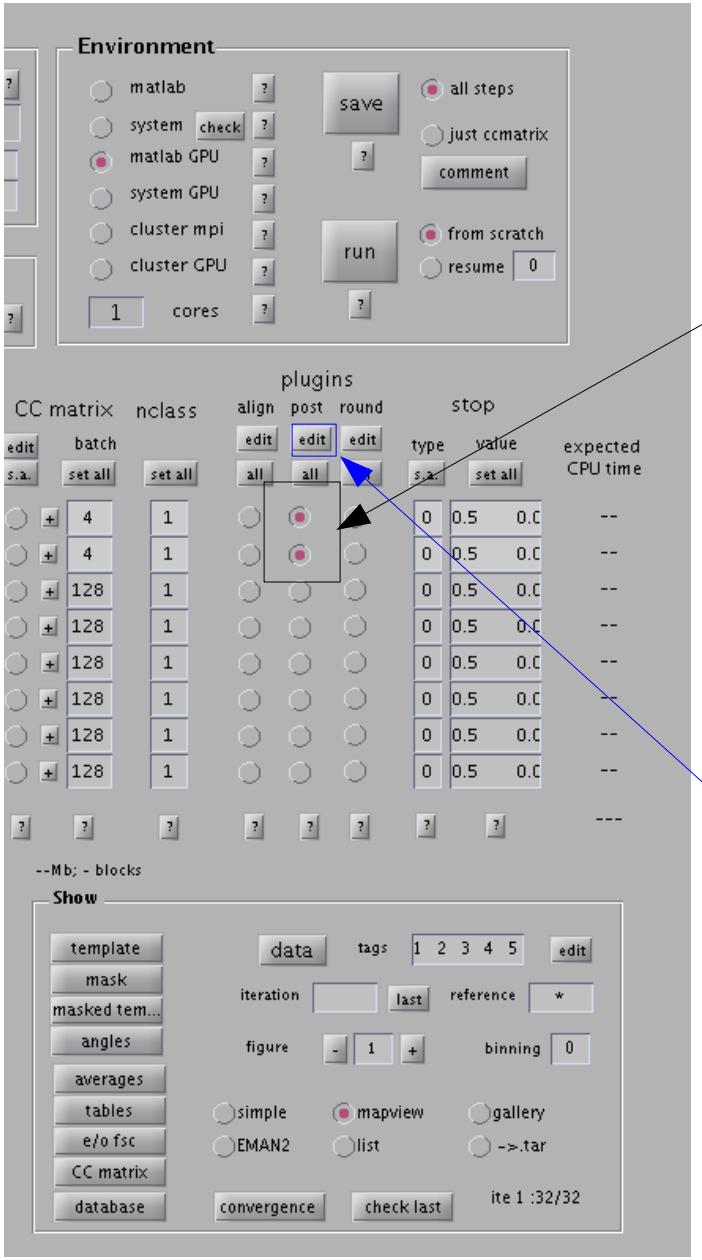
By now we have not used any real multireference approach.

If we just indicate several multireference channels, but no post-processing plugin,
Dynamo will not try to discriminate the particles by their affinity to the different templates.

Instead, all particles will be compared to all templates, and all the new averages will comprise all particles.

Thus, the two averages will contain all the particles, although with different alignment parameters.

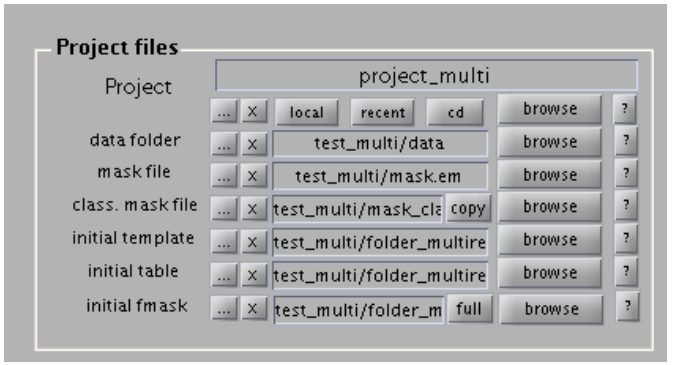
Thus, the next step is to use a plugin that considers the correlation coefficient collected by each particle against each template, and uses this information to discriminate particles.



...now let us repeat it with a real multireference police.

Open the project in the project_manager and check on the radiobuttons that activate the plugins for postprocessing.

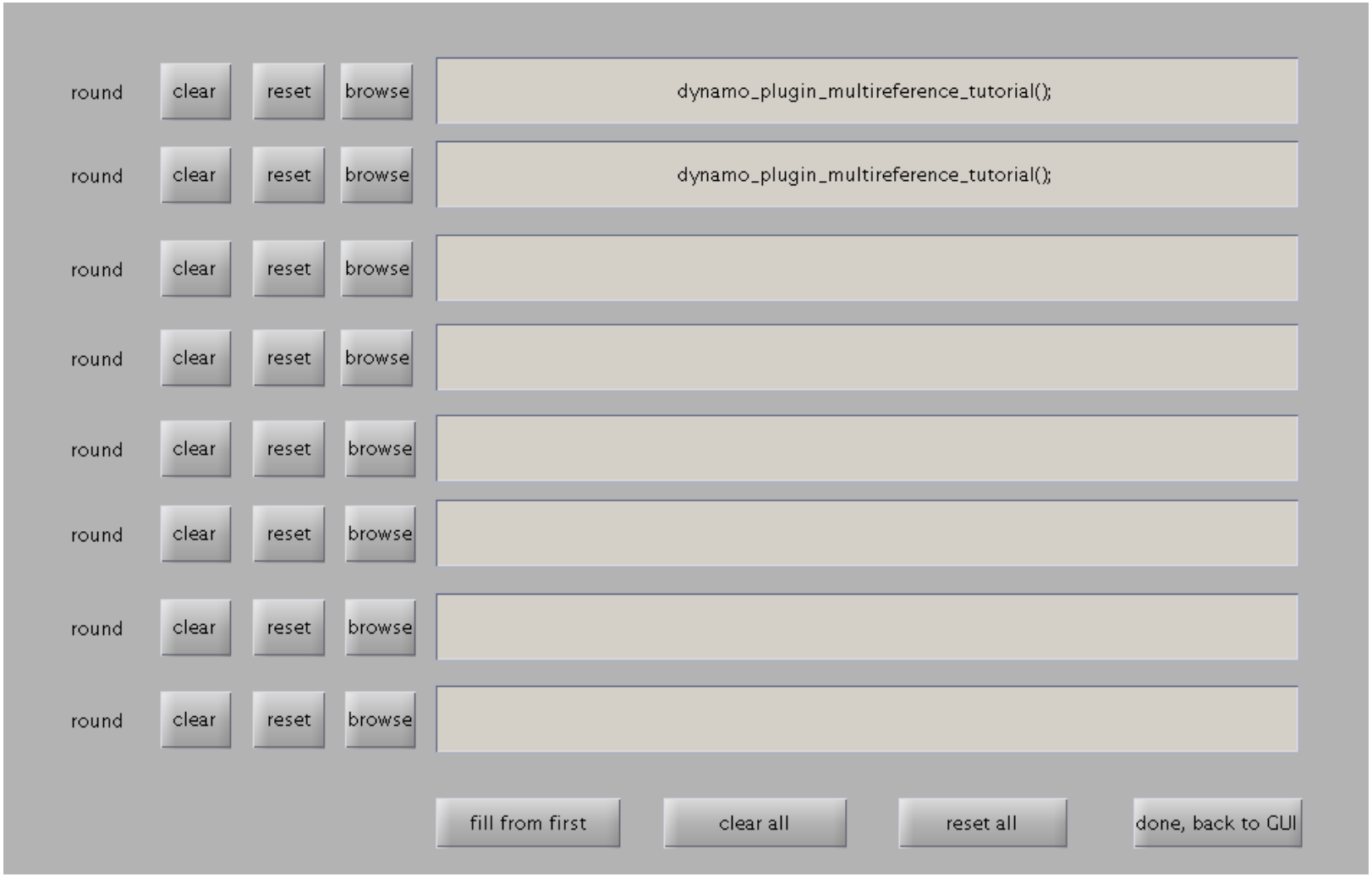
Then provide a new name for the project, save it and run it.



while your project runs, we can take a look on the syntax used to link the plugin that we have activated

Press the [edit] button: the plugin linking editor will appear.

This editor just exposes how Dynamo will invoke the plugins during its execution pipeline.
Plugins themselves are defined elsewhere, and are just executables located somewhere in the execution Path of Matlab or the operative system.

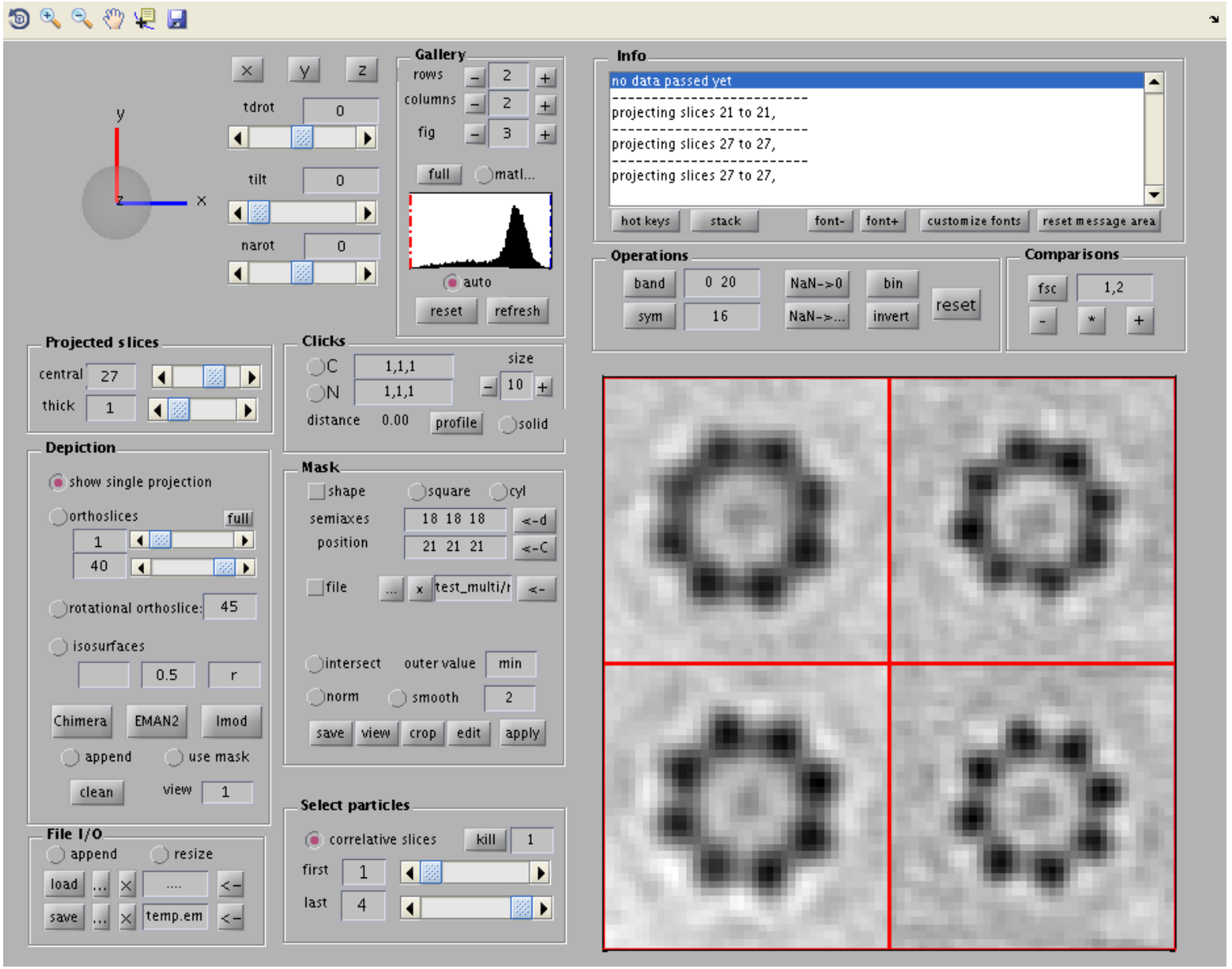


... that's it. This particular plugin does not need any parameter, so that you only pass the name of the plugin.

The () notation corresponds to Matlab notation: if you are executing a standalone version, just skip these brackets.

Now, take a look on the results on the results of the “really” multireference project we've called project_multi

using the same viewing settings as in the no multireference case for the averages...

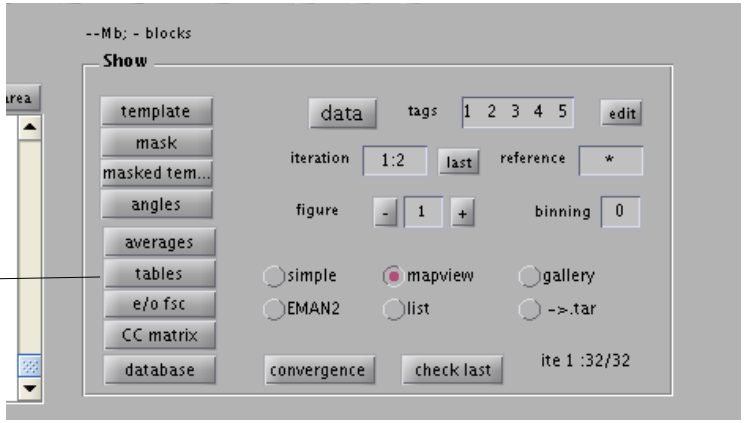


... we see that when we use the plugin the second iteration separates the two populations

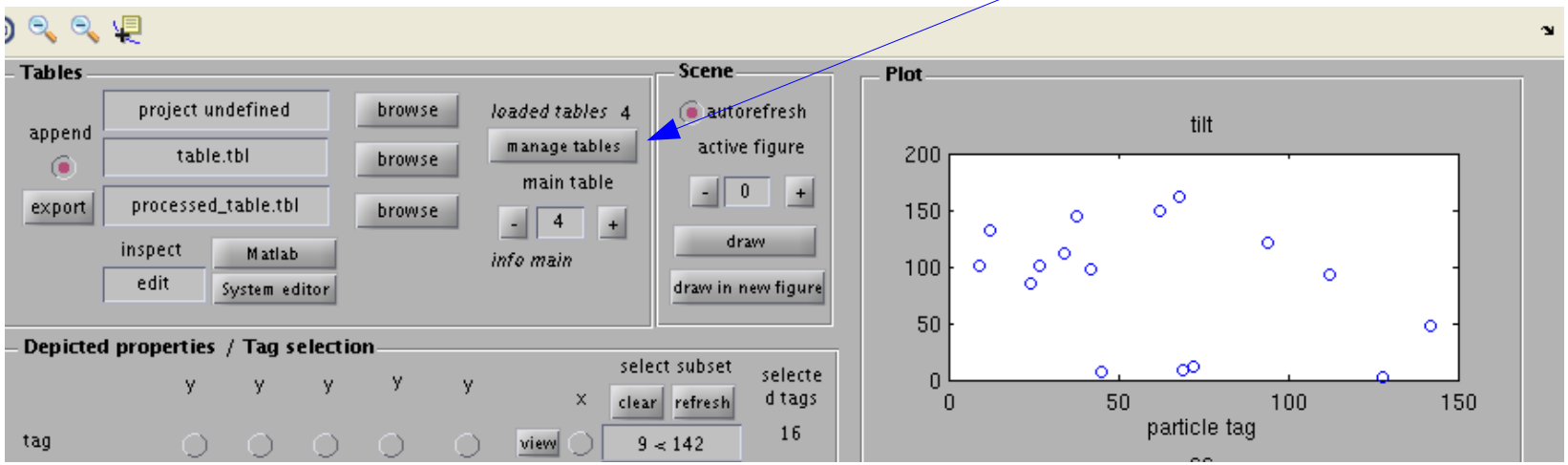
It is also instructive to take a look on the tables:

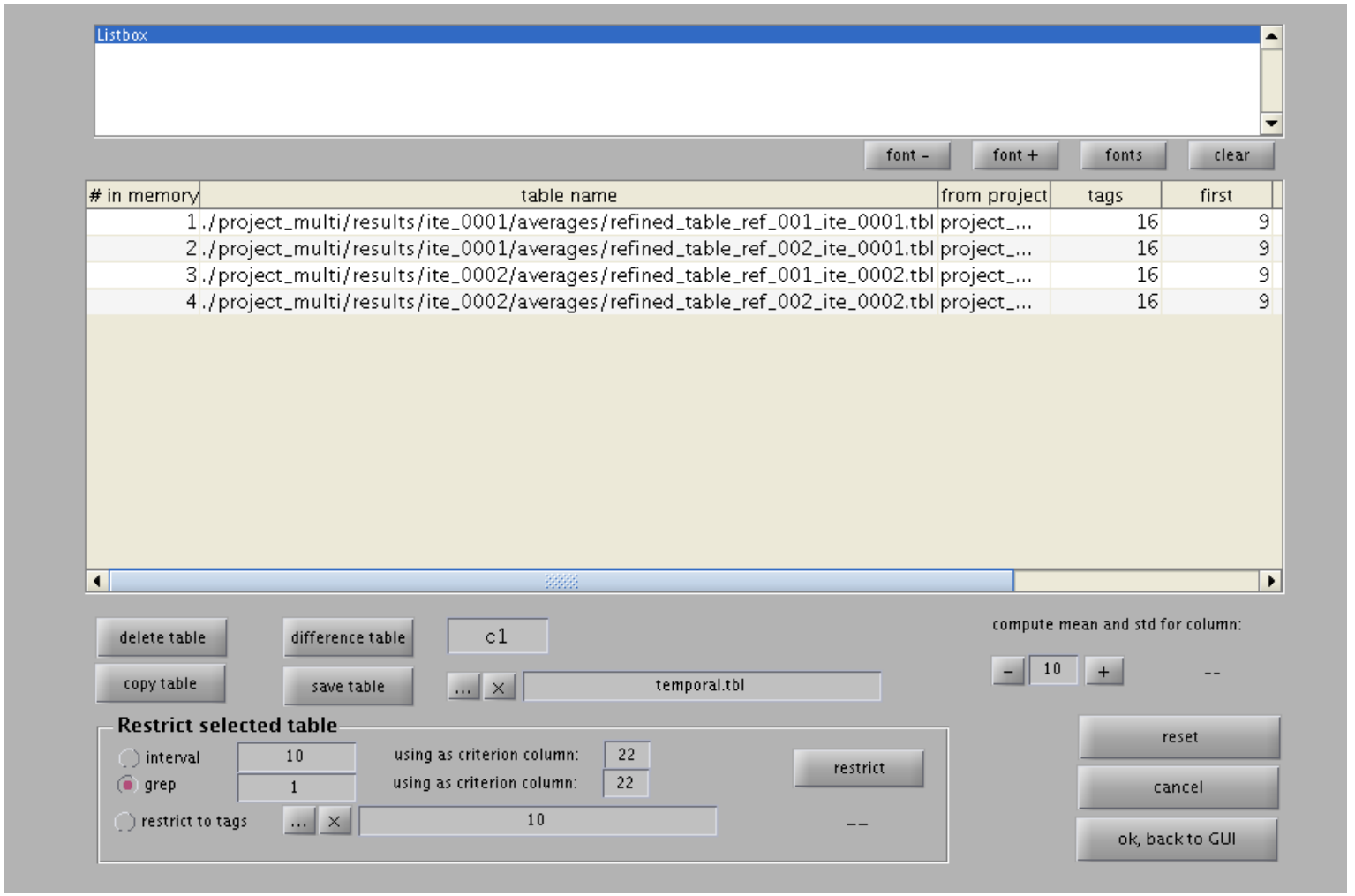
We will “cheat” using information hidden in the tables: the column 22 marks each particle with 1 or 2, signaling to which class the particle actually belongs (by construction as the tutorial tool generated them). Anyway, we want to see how the different particles behave during the iteration.

open in tableview
our four tables
(two iterations and two references)



tableview will open containing these four files. You can check the ordering in the table manager





ok, let us take a look on the last table, which corresponds to the second iteration, second reference:

use these settings:

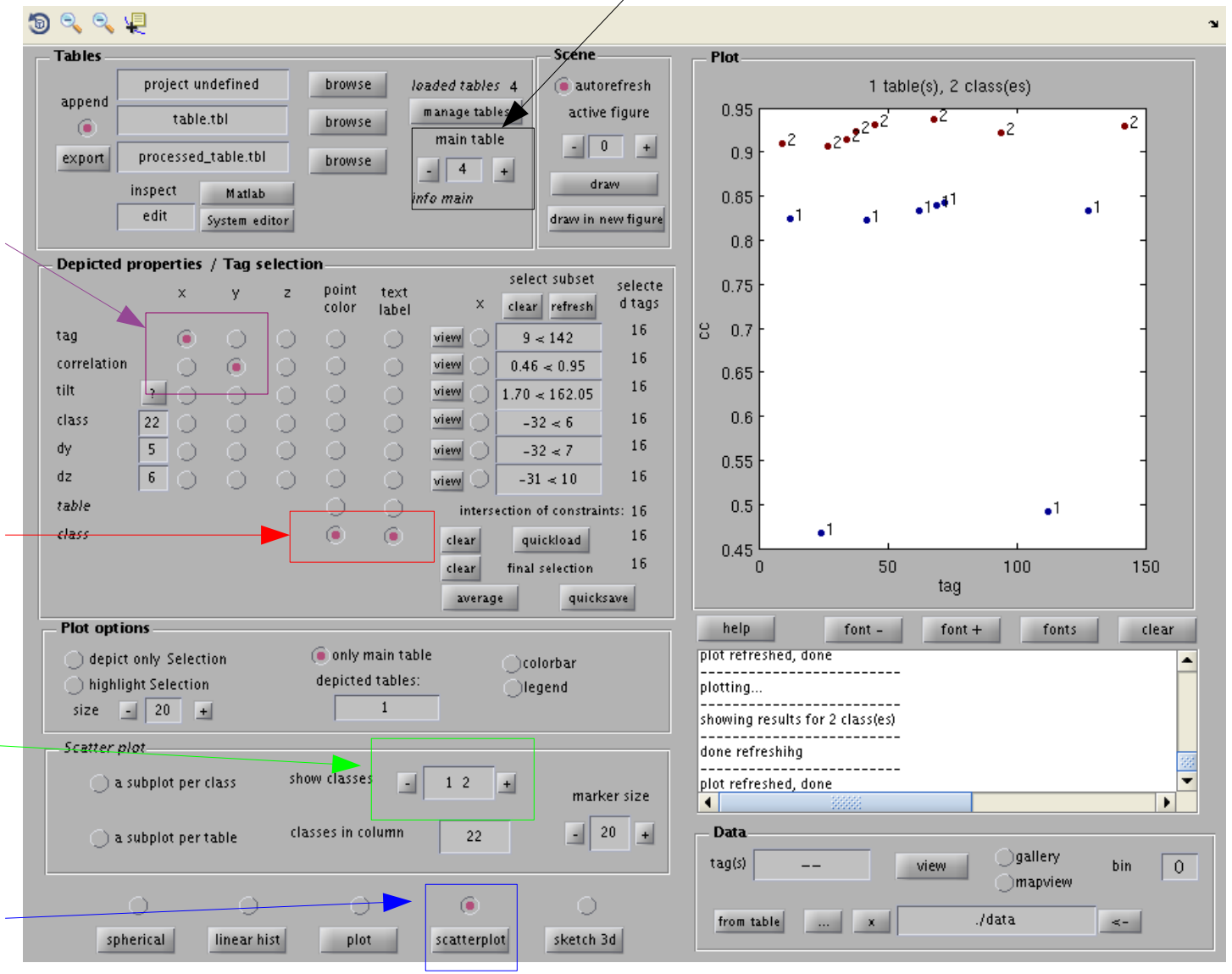
focus on table 4 (i.e. ref 2, ite 2)

depict correlation against tag

color and label depicted classes

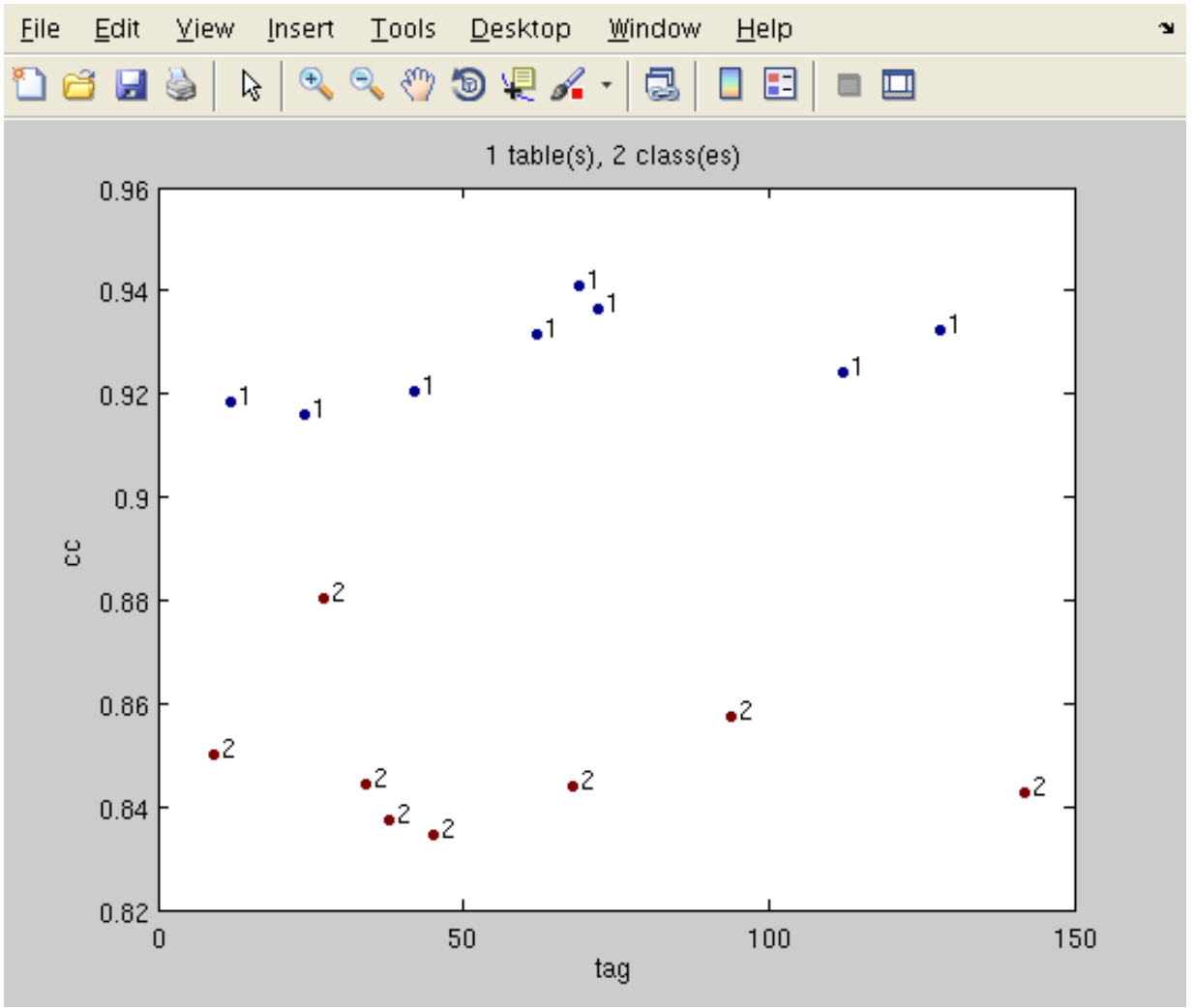
see all classes (1 and 2)

scatterplot modus

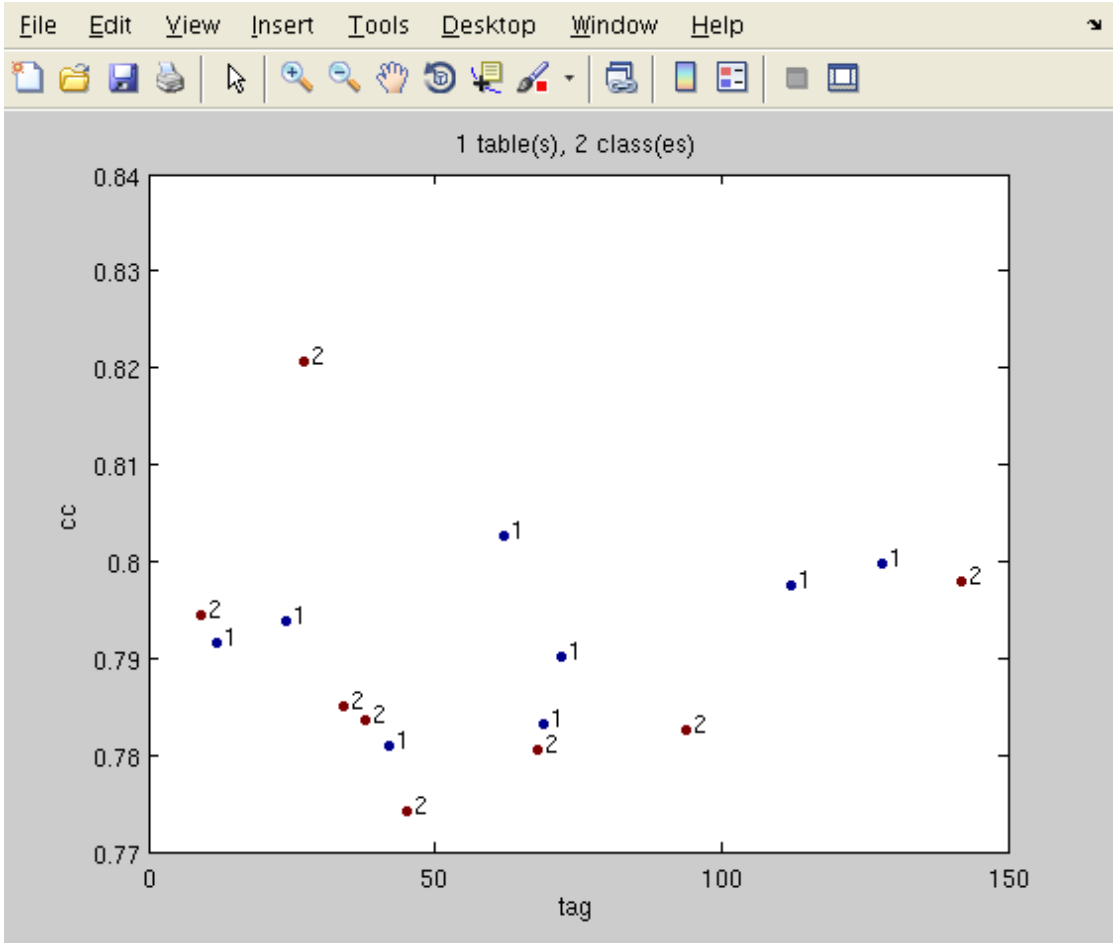


The scatter plot shows that particles actually belonging to class 2 (per construction) actually yield systematically a better scoredwhen compared to the average produced in channel 2 that particles from class 2

you can move the main table to value “3” and see that class 1 behave anologously, at least for the second iteration:



... but note that this separation is not a given! Look at the first iteration in reference 1:



class 1 and 2 have the same quantitative response to the template in channel one at this stage.
As we have seen in the previous slide, in the second iteration the template on this channel has improved and the cross correlation could actually disentangle the two populations