Picking particles:

Decorations along a filament path

Main goal:

Pick isolated particles that:

- appear irregularly distributed decorating a filament.
- approximately share a direction relative to the reference filament.

Used tools:

- Tomogram analysis: `dpreview,dslicer`
- Automatic data organization: `catalogue`
- Creation of models: model pool, model editor
- Cropping particles from tomograms into *Dynamo* data folders
- Exploration of cropping geometry: `dtplot`
- Exploration of data folders: `ddbrowse, daverage`
- Quick creation of projects: *Dynamo* wizard

We first create a synthetic data set.

```
>> dpktut.decorations.tutorial('myTest');
```

This command creates a folder containing a volume and two tables (in *Dynamo* table format)

The volume represents a slightly bent filaments, with randomly distributed motives (decorations) which are similarly oriented with relation to the filament.
All the decorations are at the same distance from the axis of the filament.

One table represents the positions of the subunits of the filament.
The second table represents the positions and orientations of the decorations.

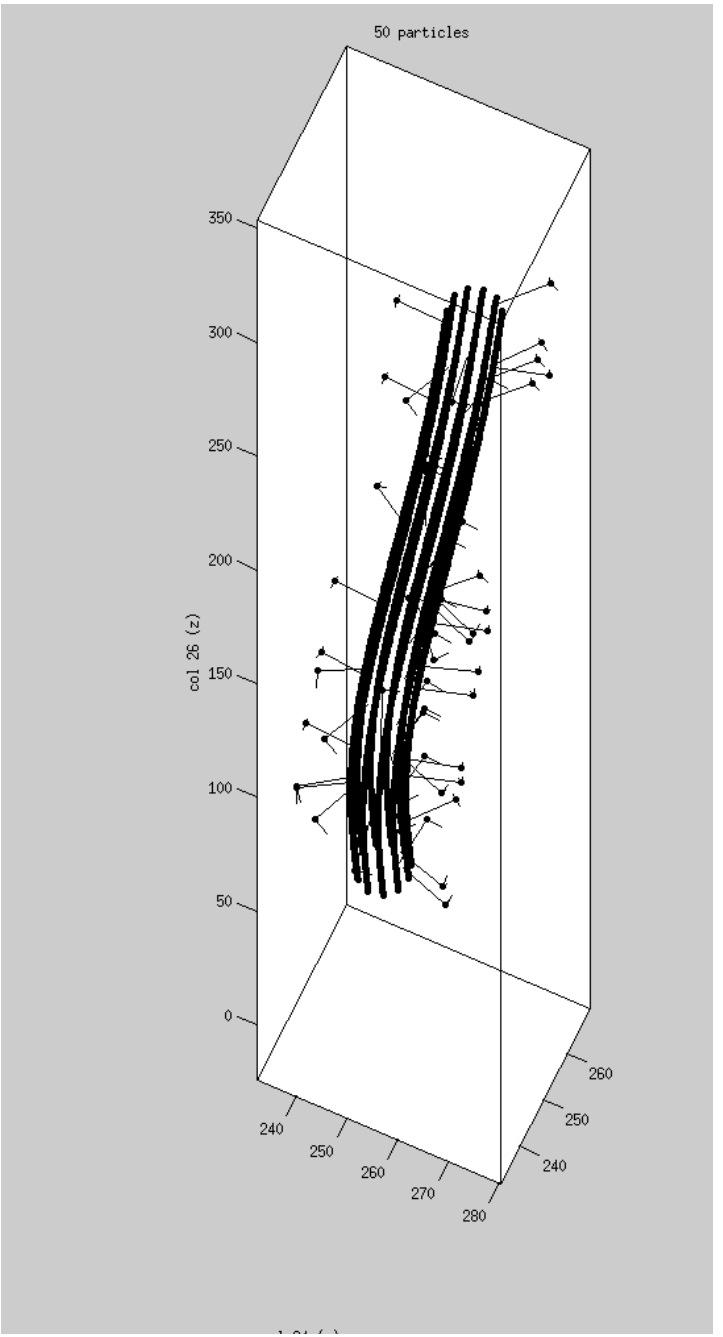
All created files are stored in “myTest”

```
....
A total of 46 particles succesfully placed in volume of dimensions 512x512x300
[tutorial] Applying missing wedge.
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Table representing the filament:
myTest/subunits.tbl
Table representing the decorations:
myTest/decorations.tbl
Suggested visualization:
figure;hold on
dtplot myTest/subunits.tbl -pf positions
dtplot myTest/decorations.tbl -profile oriented_positions
axis equal
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Volume with filament and irregular decorations written in:
myTest/volume.em
to enter it into a catalogue and start analyzing the volume type:
dpreview('myTest/volume.em');
------------------------------------------------------------
```

The tutorial gives us a suggestion on how we can visualize the geometry that it just produced.

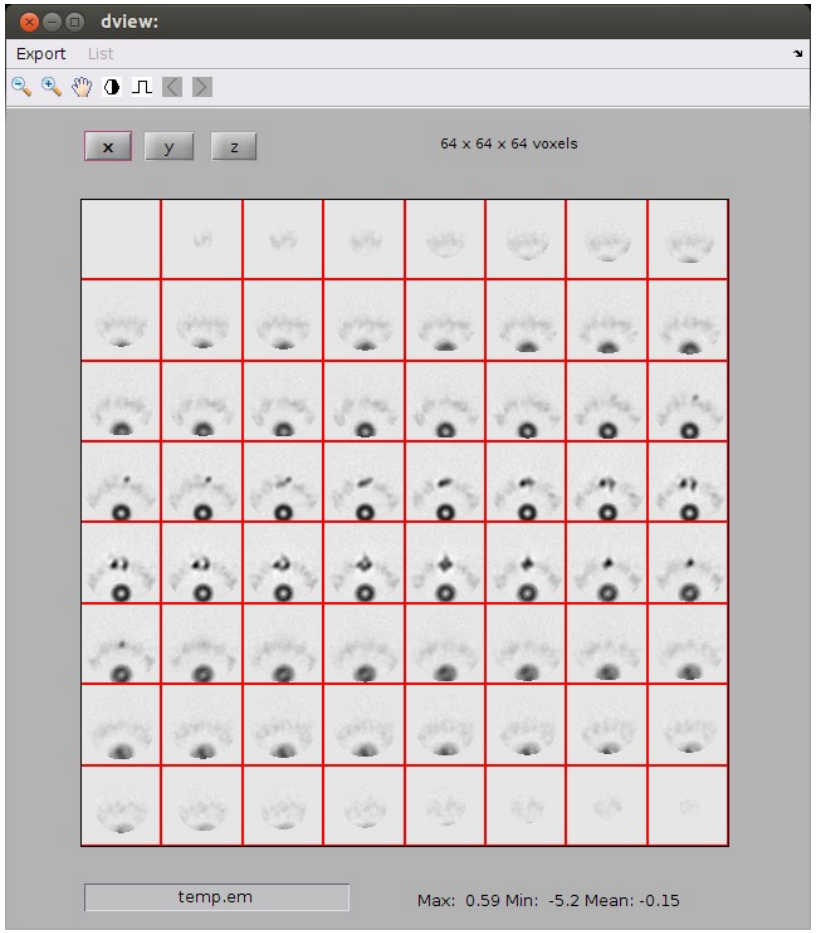
One table models the subunits that constitute the filament: `myTest/subunits.tbl`
The other are the positions of the decorations: `myTest/decorations.tbl`

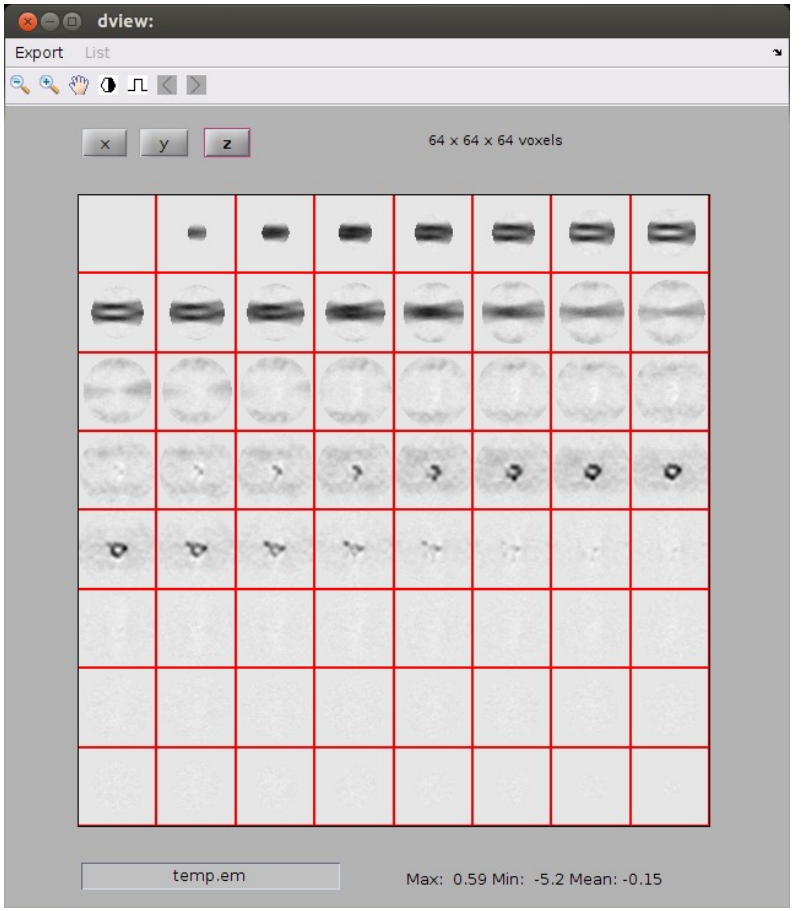
Graphical output of `dtplot`
for both tables on the same figure:



We can average together all the particles that we have planted inside the simulated tomogram

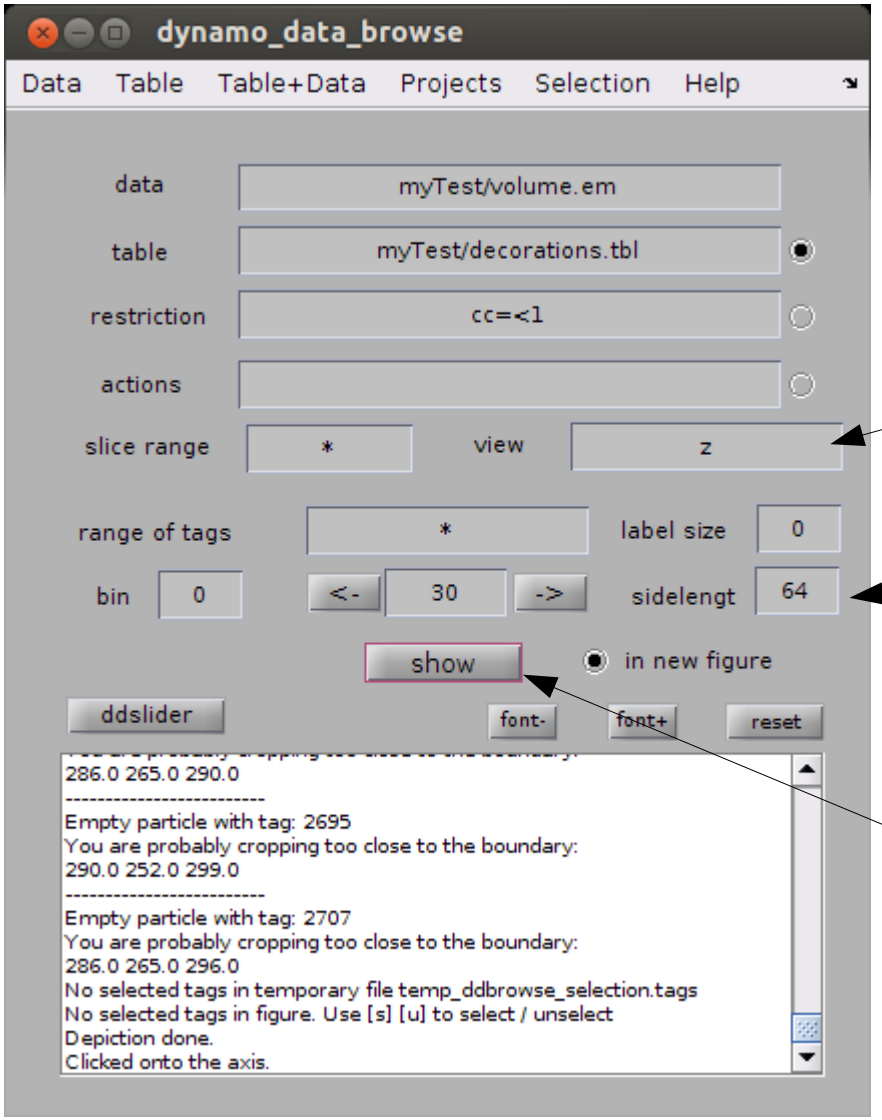
```
daverage myTest/volume.em -t myTest/decorations.tbl -sidelength 64 -ws a;
dview(a);
```





We can take a look onto the particles themselves with `ddbrowse`:

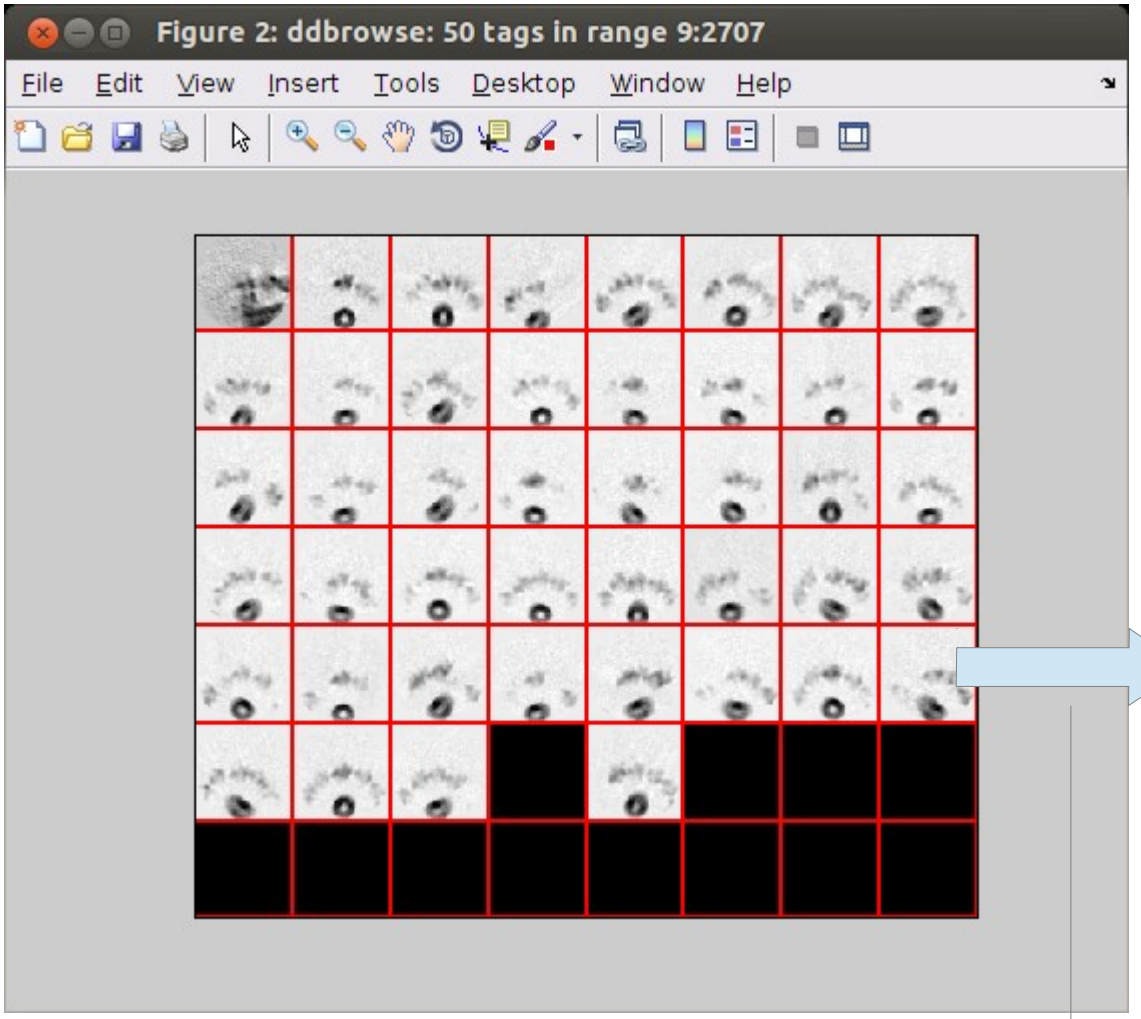
```
ddbrowse -d myTest/volume.em -t myTest/decorations.tbl
```

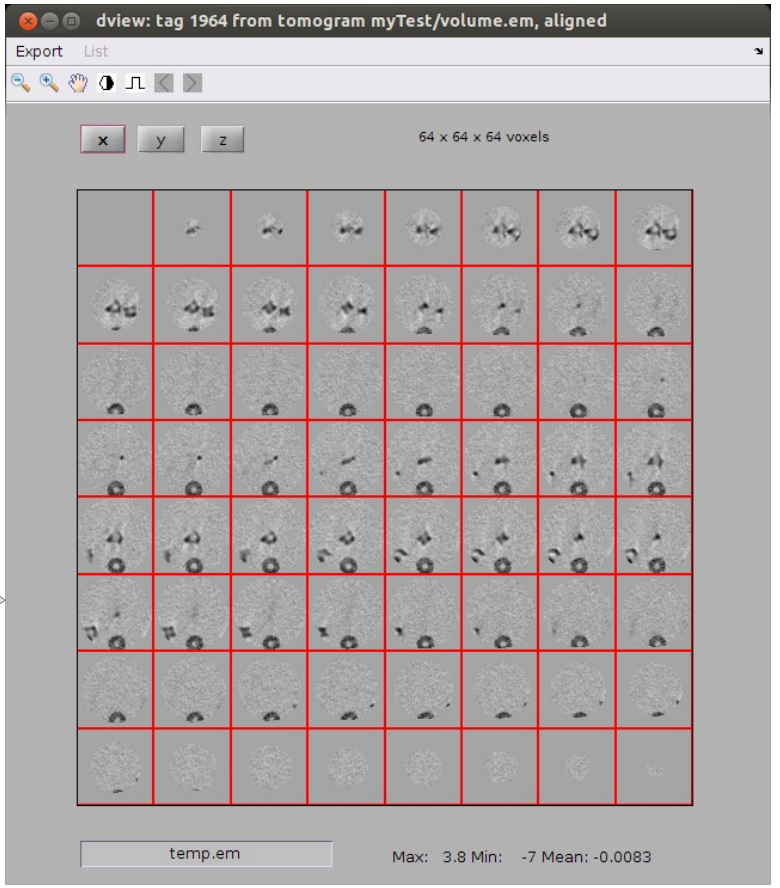


selects a viewing direction

selects a sidelength to crop each subtomogram

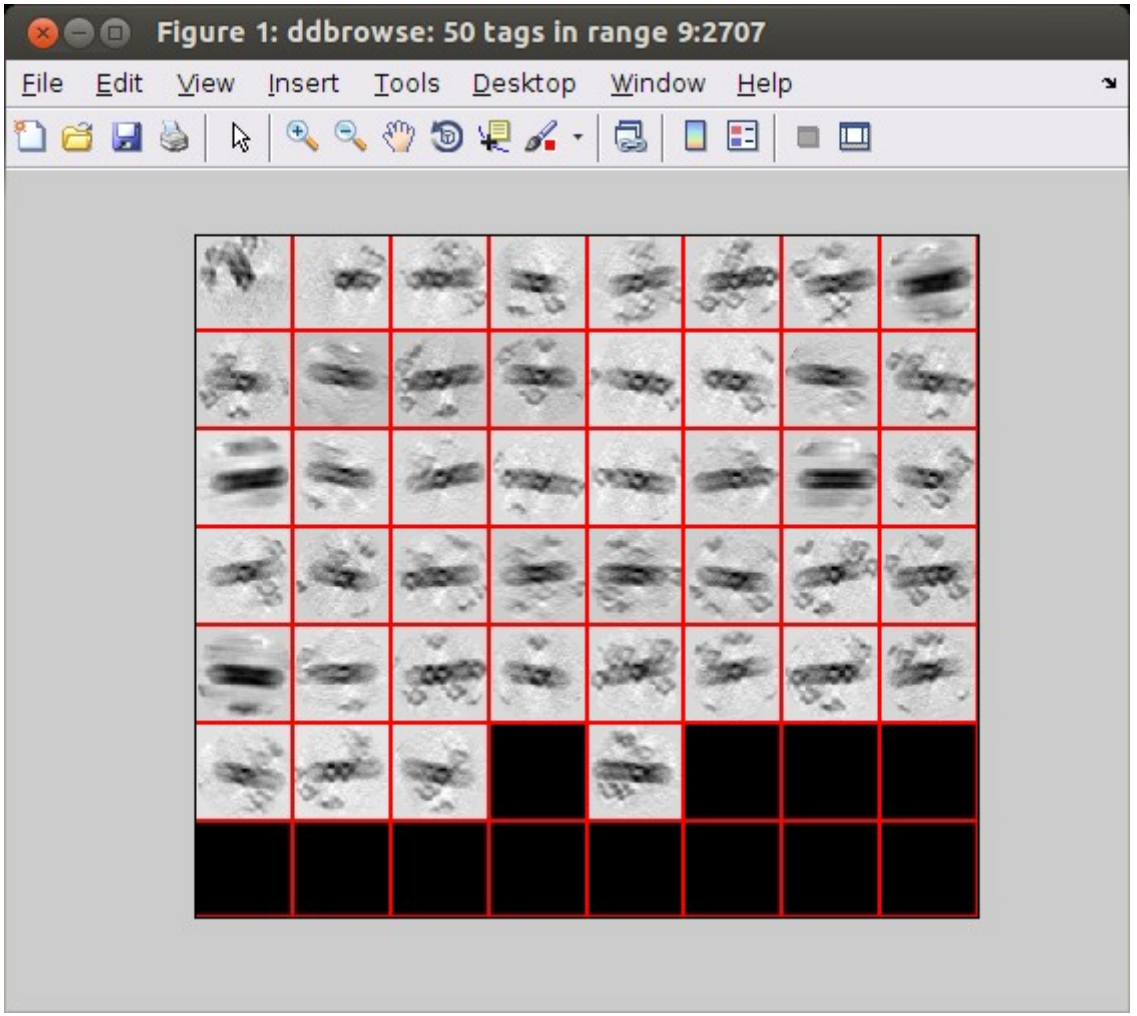
Press [show] to see theparticles





x view (projection) of all the particles cropped from the volume

(click on one of them to access an individual particle)



z projection view: the direction of the filament (roughly along x) varies slightly from particle to particle

```
....
A total of 46 particles succesfully placed in volume of dimensions 512x512x300
[tutorial] Applying missing wedge.
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Table representing the filament:
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Suggested visualization:
figure;hold on
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axis equal
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
Volume with filament and irregular decorations written in:
myTest/volume.em
to enter it into a catalogue and start analyzing the volume type:
dpreview('myTest/volume.em');
----------------------------------------------------------------
```
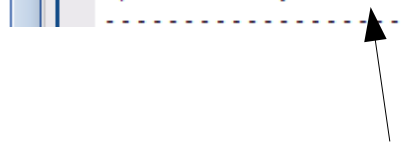
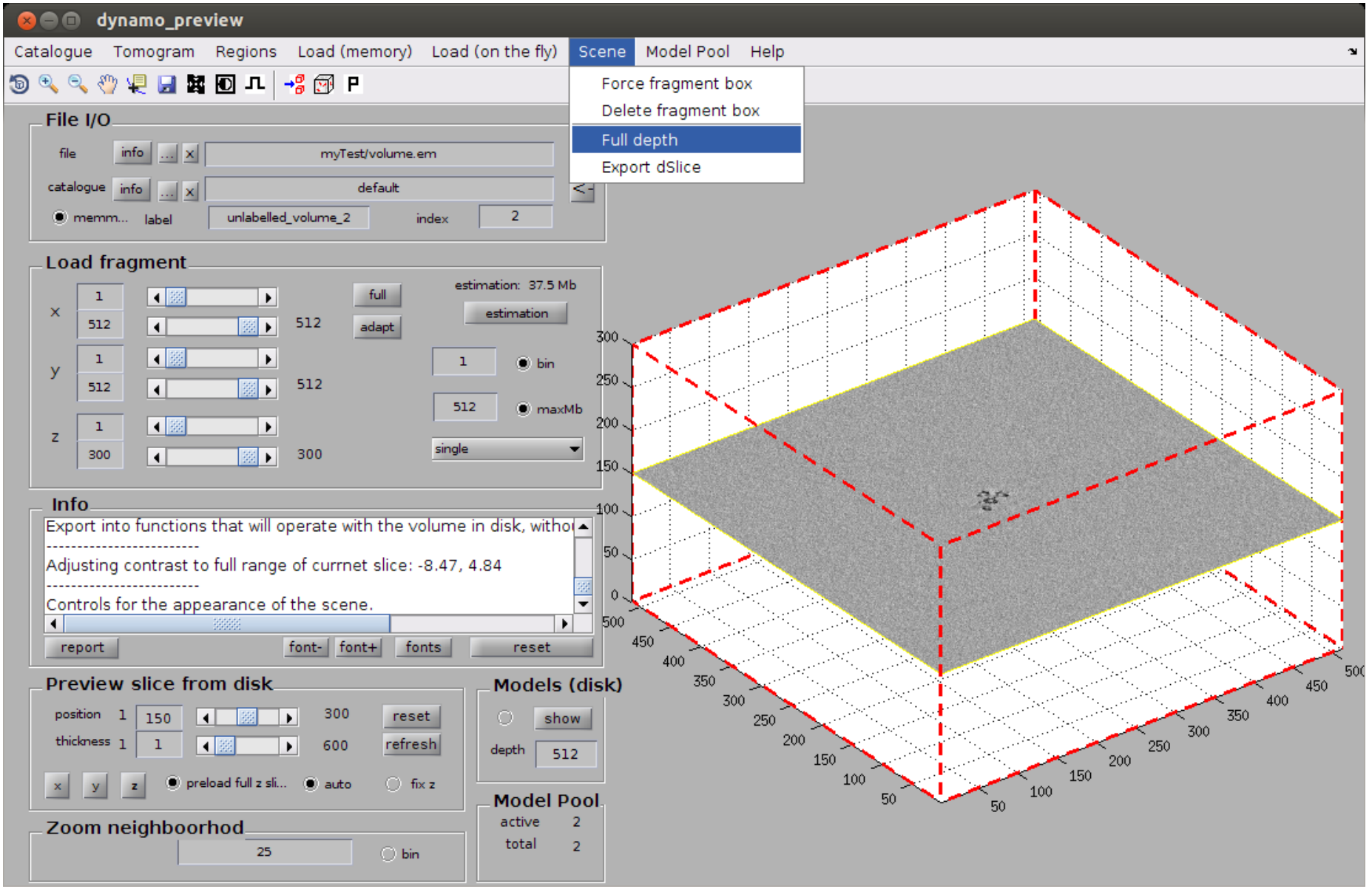
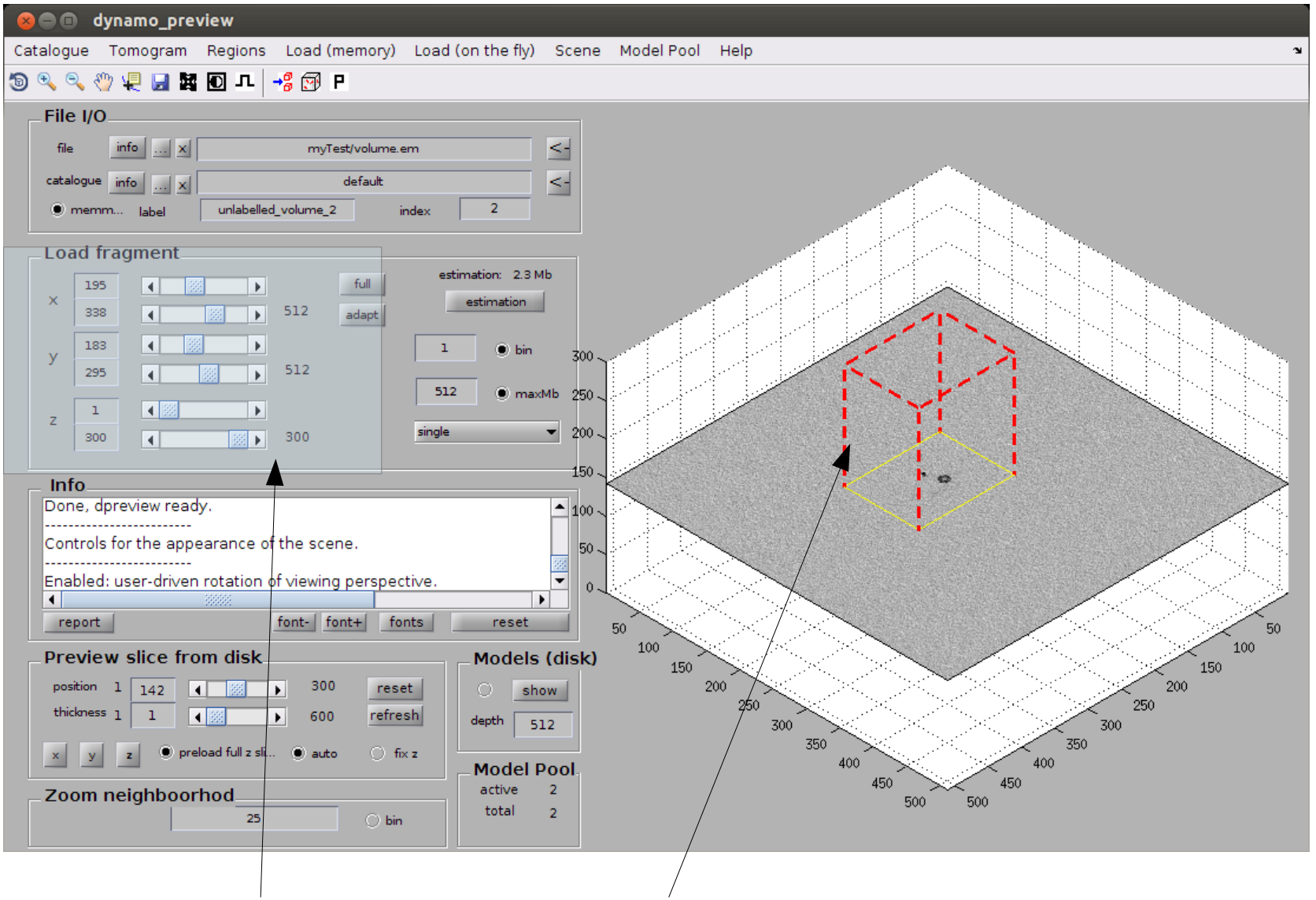



If we follow the instructions we can open the preview browser onto the volume.

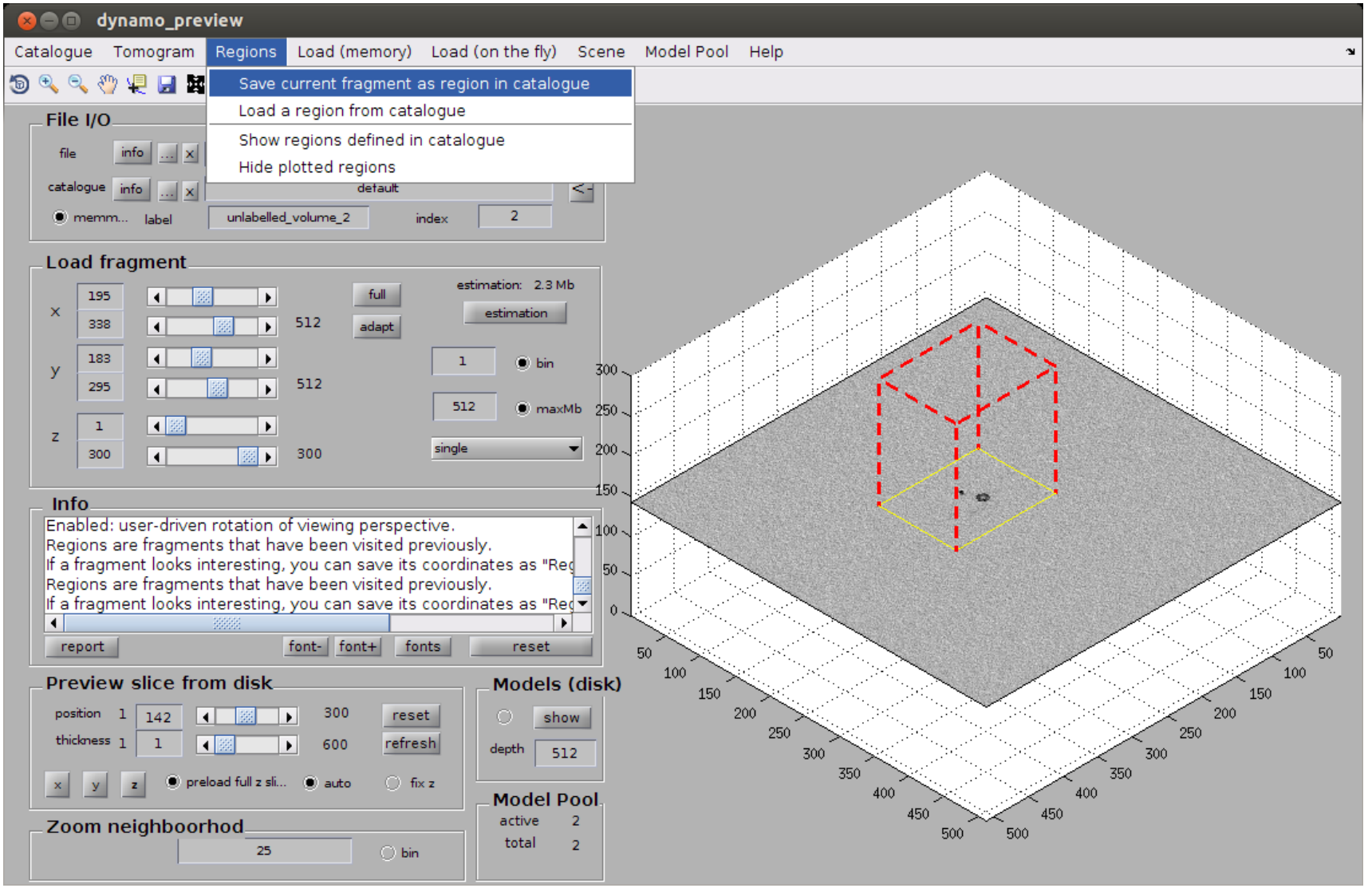

dynamo_preview
Catalogue
Tomogram
Regions
Load (memory)
Load (on the fly)
Scene
Model Pool
Help
Force fragment box
Delete fragment box
Full depth
Export dSlice
File I/O
file
info
myTest/volume.em
catalogue
default
memm...
label
unlabelled_volume_2
index
2
Load fragment
x
y
z
full
adapt
estimation: 37.5 Mb
estimation
bin
maxMb
single
Info
Export into functions that will operate with the volume in disk, witho
Adjusting contrast to full range of currnet slice: -8.47, 4.84
Controls for the appearance of the scene.
report
font-
font+
fonts
reset
Preview slice from disk
position
thickness
reset
refresh
preload full z sli...
auto
fix z
Zoom neighboorhod
bin
Models (disk)
show
depth
512
Model Pool
active
total

Instead of sending the full tomogram to another browser that allows flexible annotation of models, we can choose an area of interest to be sent to that browser

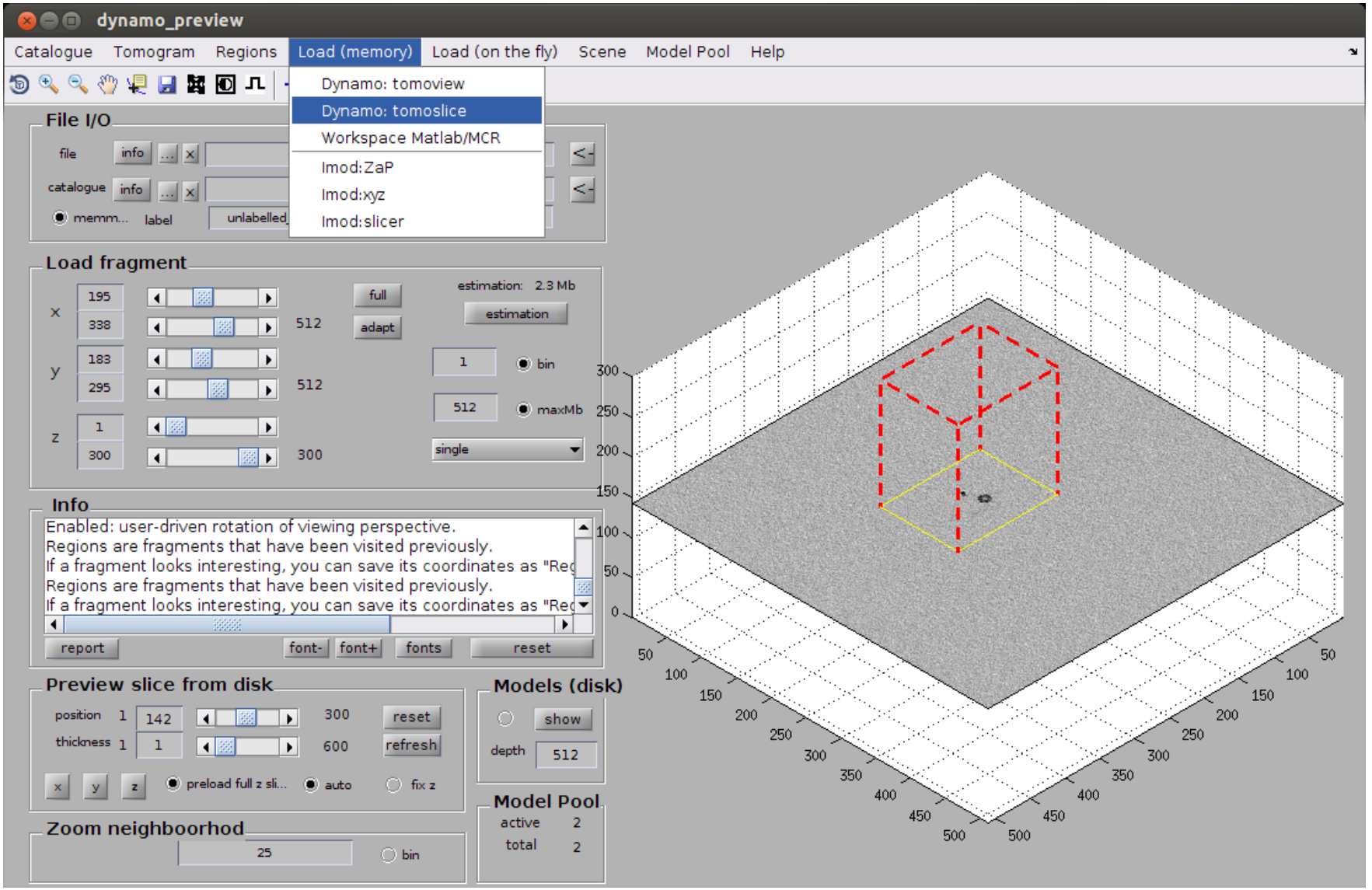


Control the extent of the tomogram aread that will be sent to the main memory

Note that it is possible to save the extents of the region of interest, anticipiating future visits to this area. By default the region extents will be saved into the catalogue that has been already defined by `dpreview`



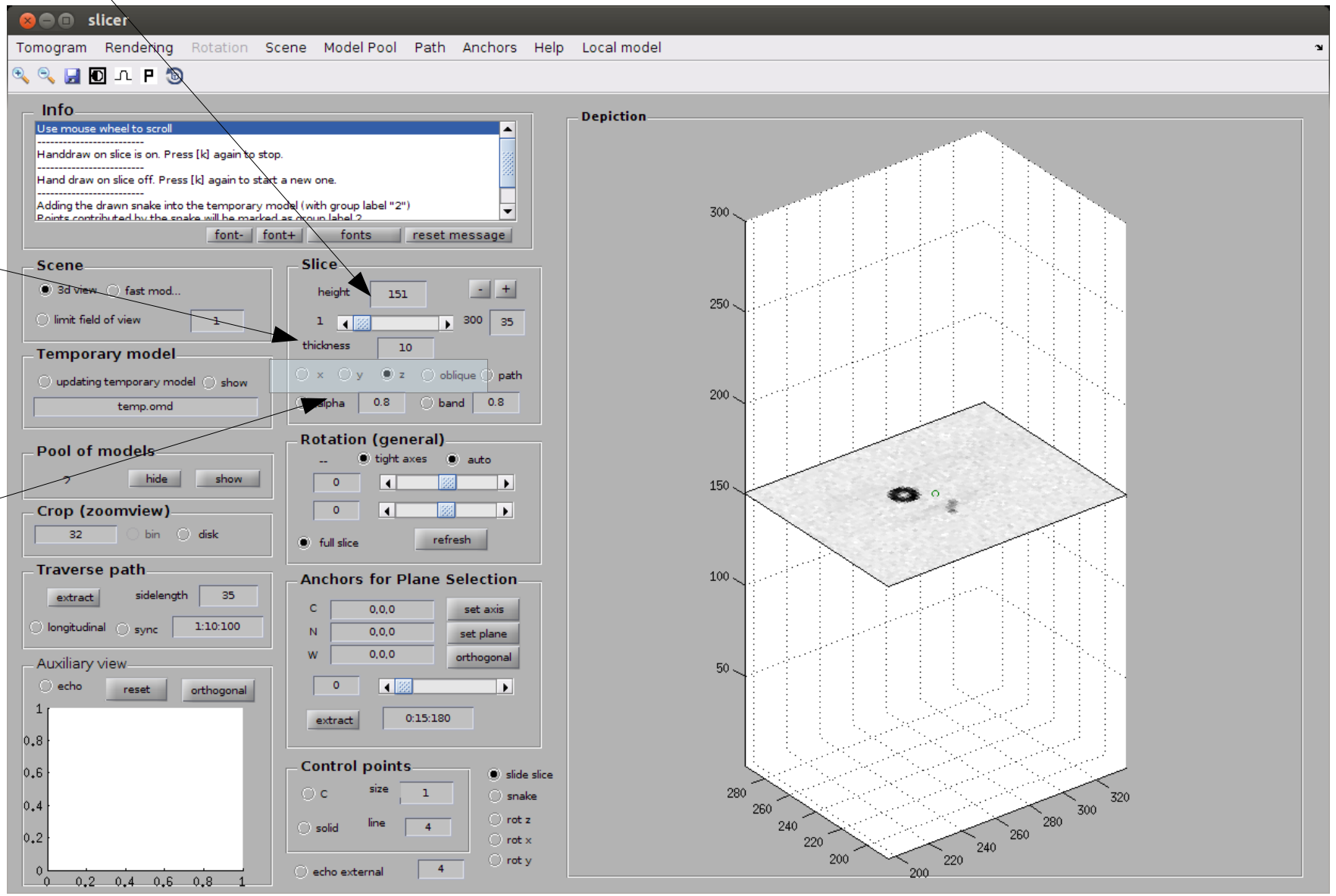
We invoke now `tomoslice`, a browser that produces oblique views and is thus specially suited for annotations on filaments.

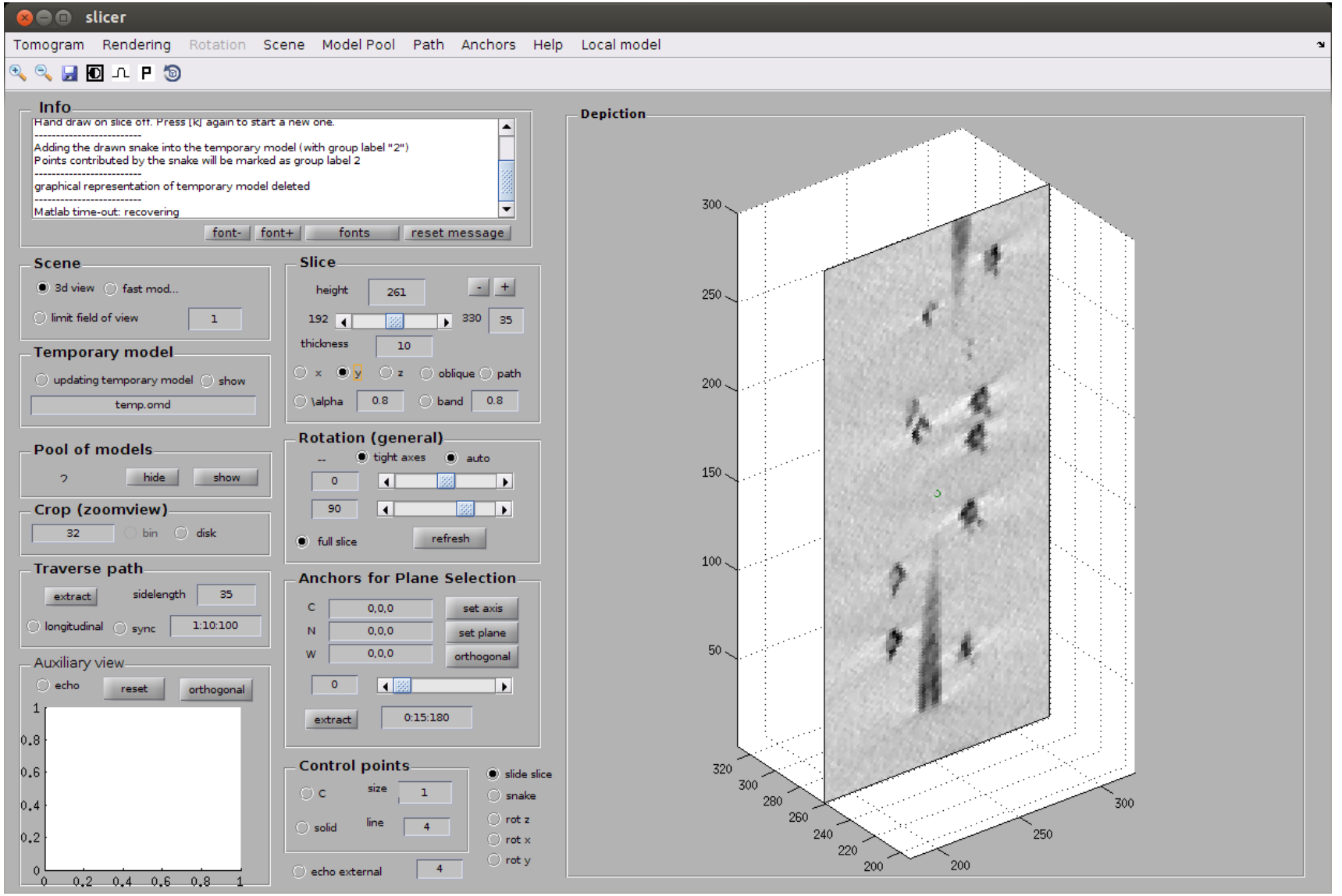


dslicer shows [at first] a single slice from the fragment currently in memory.
Three ways to control the position of the current slide:
1: dragging it with the mouse
2: with the scrollwheel
3: with this slider

projected slices represented in shown slice

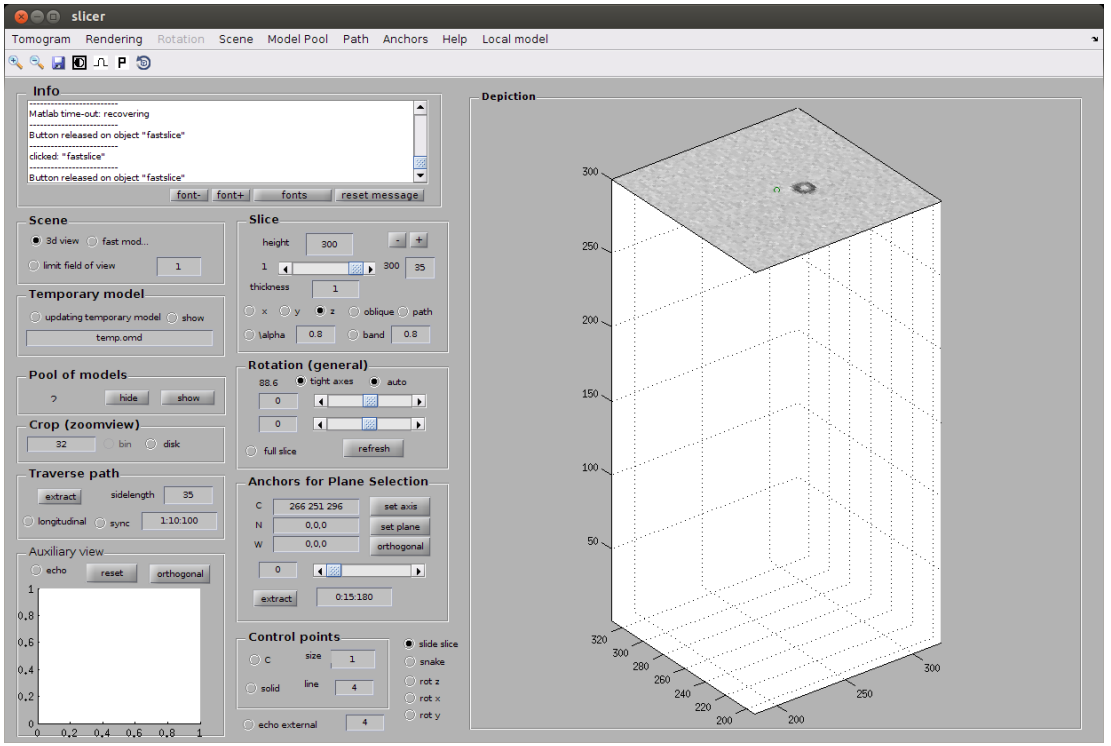
selects slice orientation

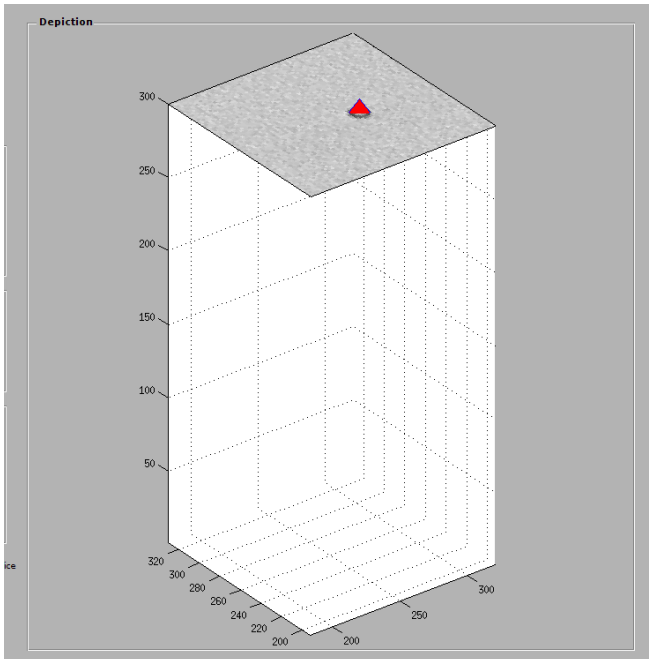



slicer
Tomogram
Rendering
Rotation
Scene
Model Pool
Path
Anchors
Help
Local model
Info
Hand draw on slice off. Press [k] again to start a new one.

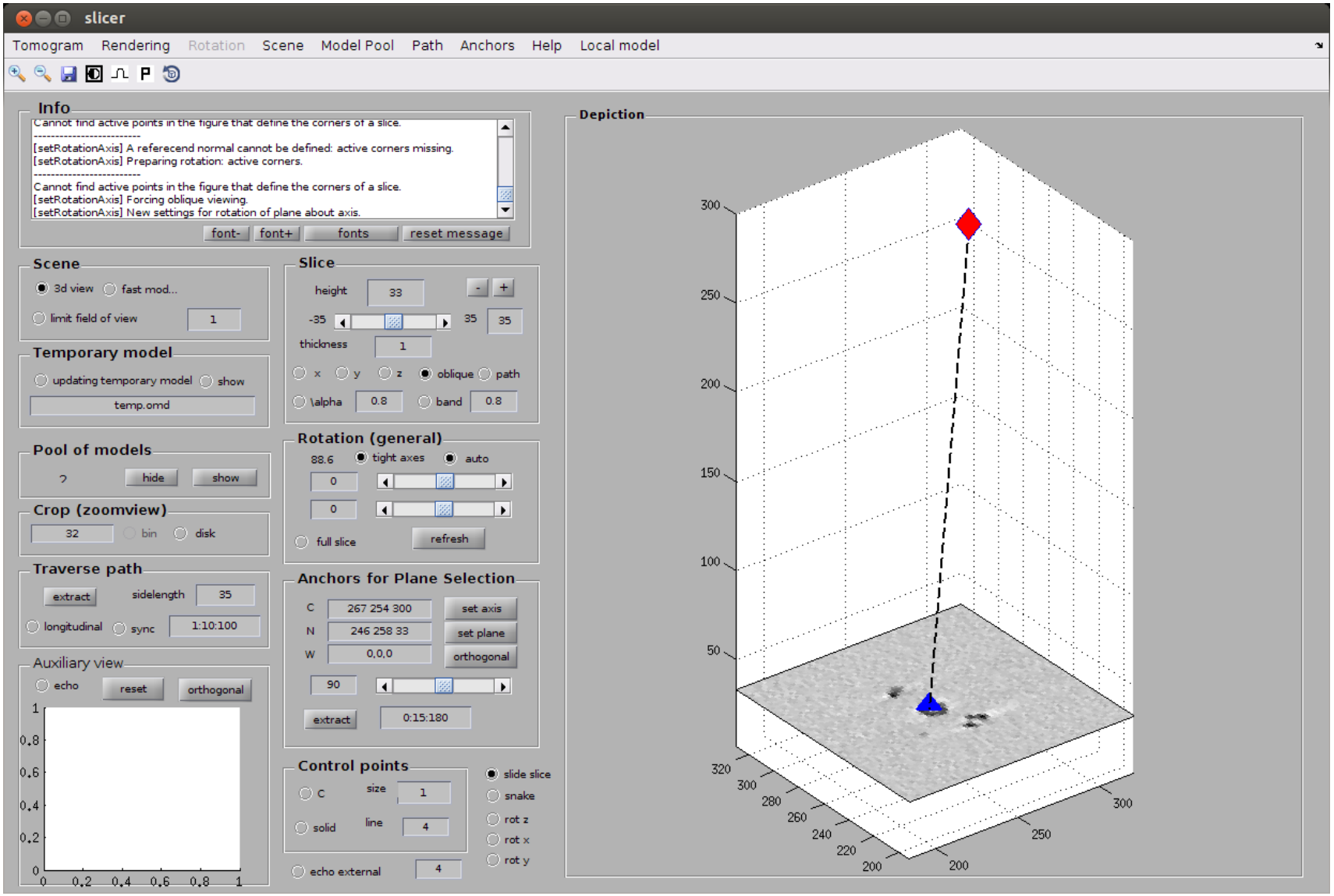
Adding the drawn snake into the temporary model (with group label "2")
Points contributed by the snake will be marked as group label 2
---
graphical representation of temporary model deleted

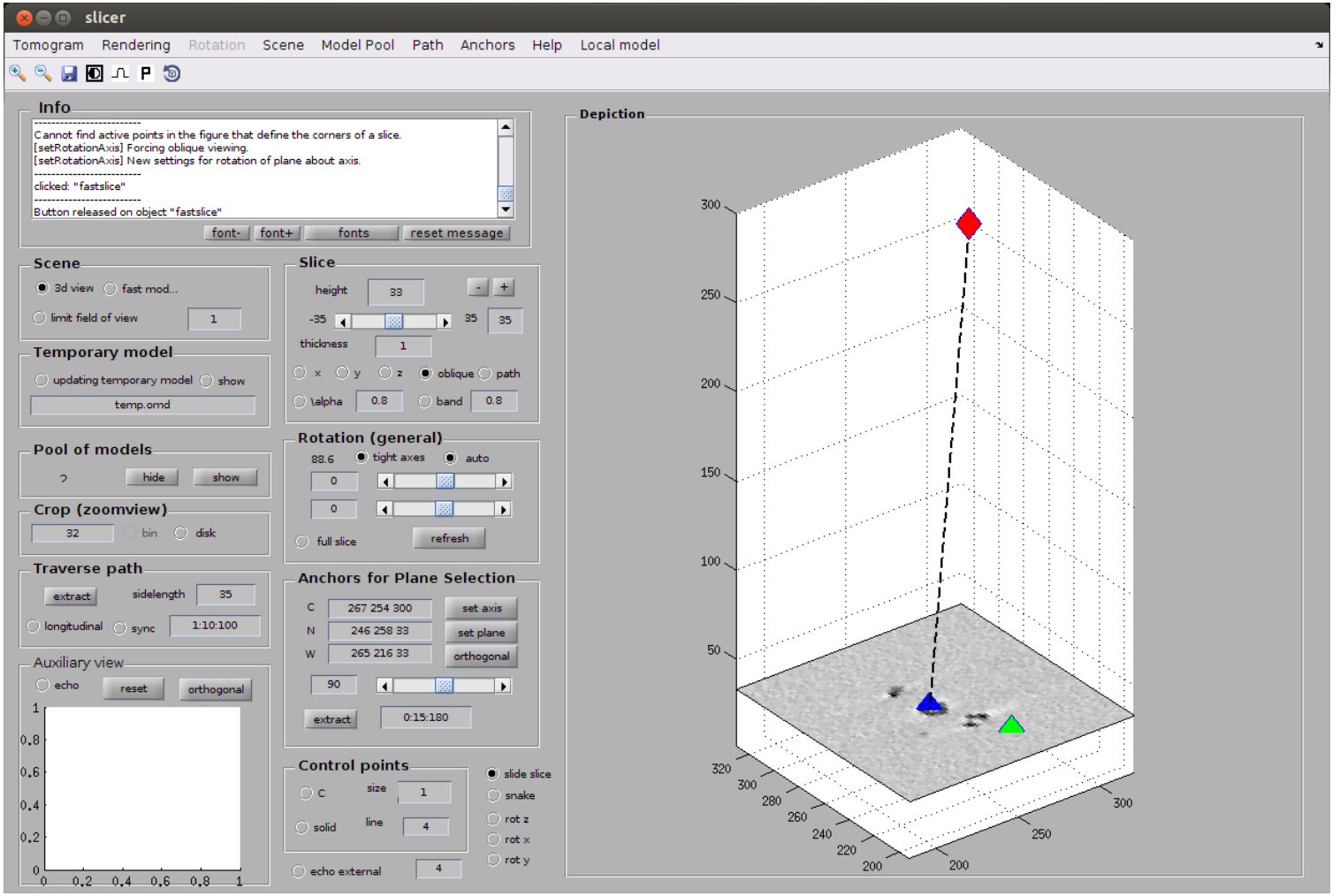
Matlab time-out: recovering
font-
font+
fonts
reset message
Scene
3d view
fast mod...
limit field of view
1
Temporary model
updating temporary model
show
temp.omd
Pool of models
?
hide
show
Crop (zoomview)
32
bin
disk
Traverse path
extract
sidelength
35
longitudinal
sync
1:10:100
Auxiliary view
echo
reset
orthogonal
Slice
height
261
192
330
35
thickness
10
x
y
z
oblique
path
\alpha
0.8
band
0.8
Rotation (general)
tight axes
auto
0
90
full slice
refresh
Anchors for Plane Selection
C
0,0,0
set axis
N
0,0,0
set plane
W
0,0,0
orthogonal
0
extract
0:15:180
Control points
C
size
1
solid
line
4
echo external
4
slide slice
snake
rot z
rot x
rot y
Depiction


slicer
Tomogram Rendering Rotation Scene Model Pool Path Anchors Help Local model
Info
Matlab time-out: recovering
Button released on object "fastslice"
clicked: "fastslice"
Button released on object "fastslice"
font- font+ fonts reset message
Scene
3d view fast mod...
limit field of view 1
Temporary model
updating temporary model show
temp.omd
Pool of models
hide show
Crop (zoomview)
32 bin disk
Traverse path
extract sidelength 35
longitudinal sync 1:10:100
Auxiliary view
echo reset orthogonal
Slice
height 300
thickness 1
x y z oblique path
alpha 0.8 band 0.8
Rotation (general)
tight axes auto
full slice refresh
Anchors for Plane Selection
C 266 251 296 set axis
N 0,0,0 set plane
W 0,0,0 orthogonal
extract 0:15:180
Control points
C size 1
solid line 4
echo external 4
slide slice
snake
rot z
rot x
rot y
Depiction

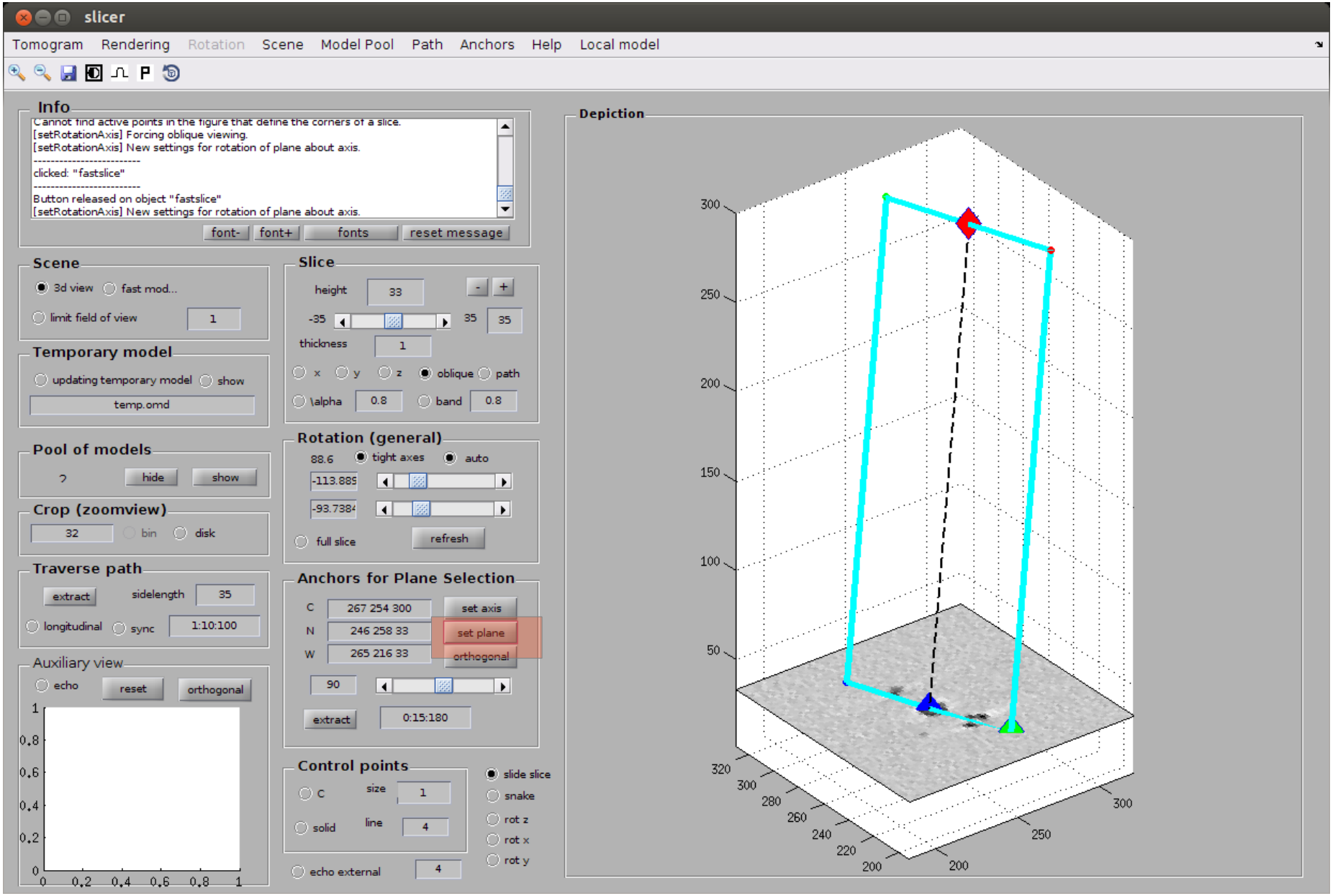

Depiction

Then, look for the other end of the filament and press [2] to create the “North” marker (blue romboid)

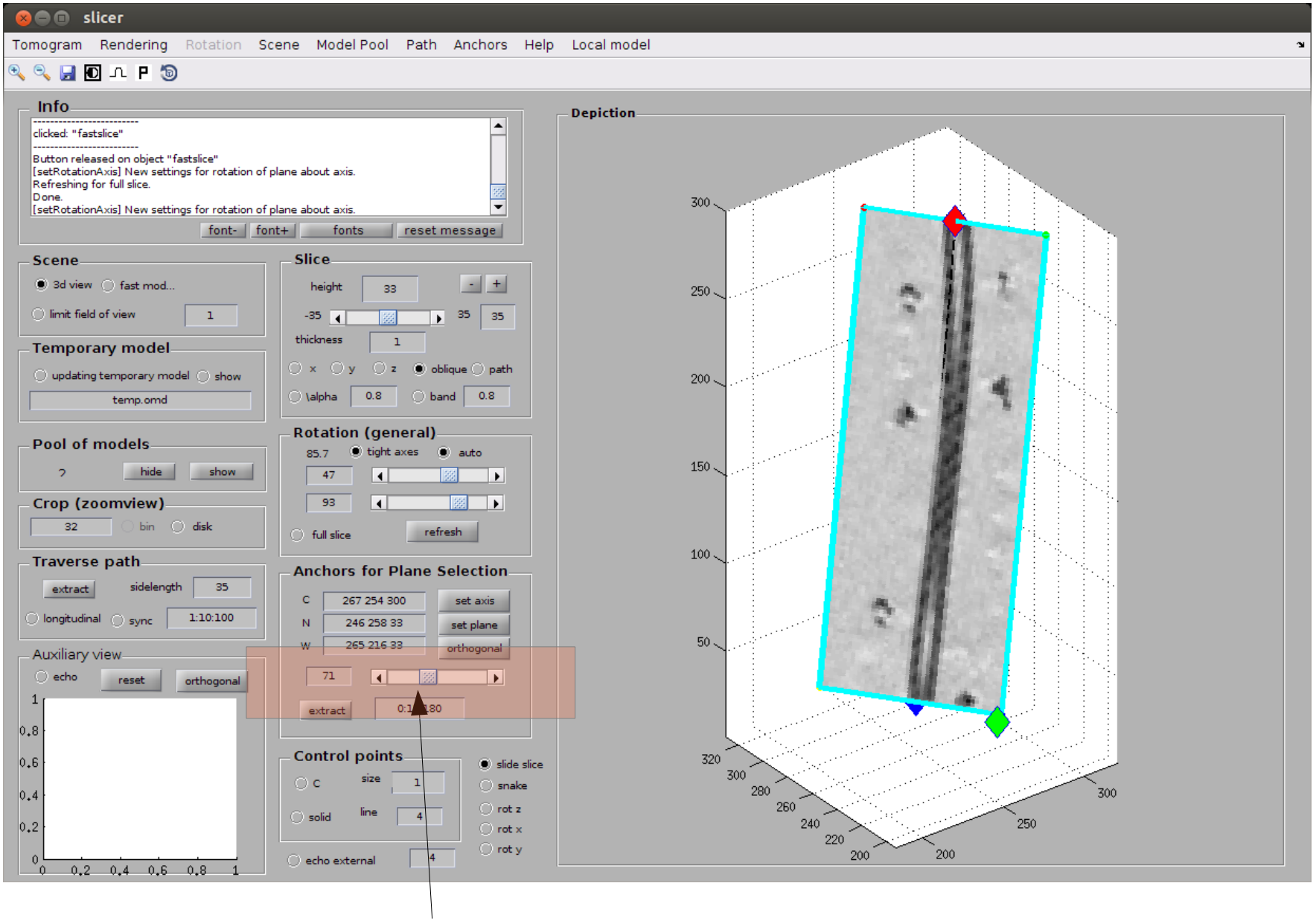




You can press [3] on the same slice to mark the extent of the area around the filament that we consider of interest.

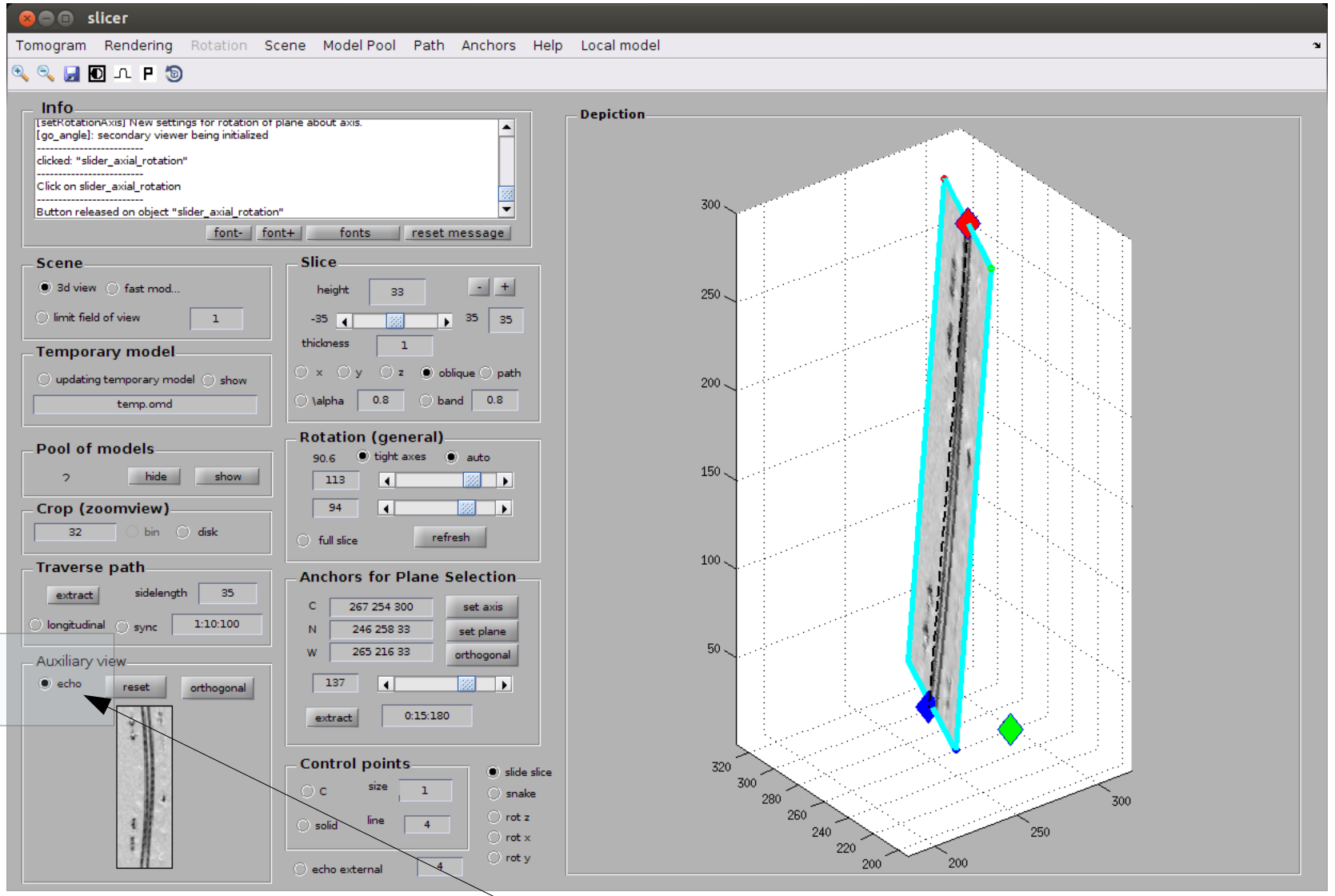


Then, [set plane] creates a section with the geometry of the points that you selected.

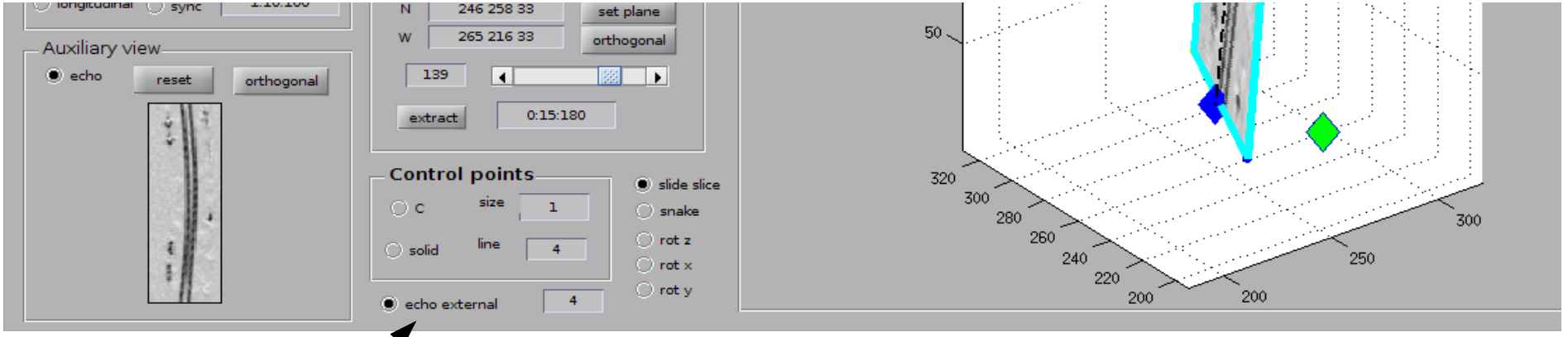


Now you can navigate a plane that rotates around the axis of the filament

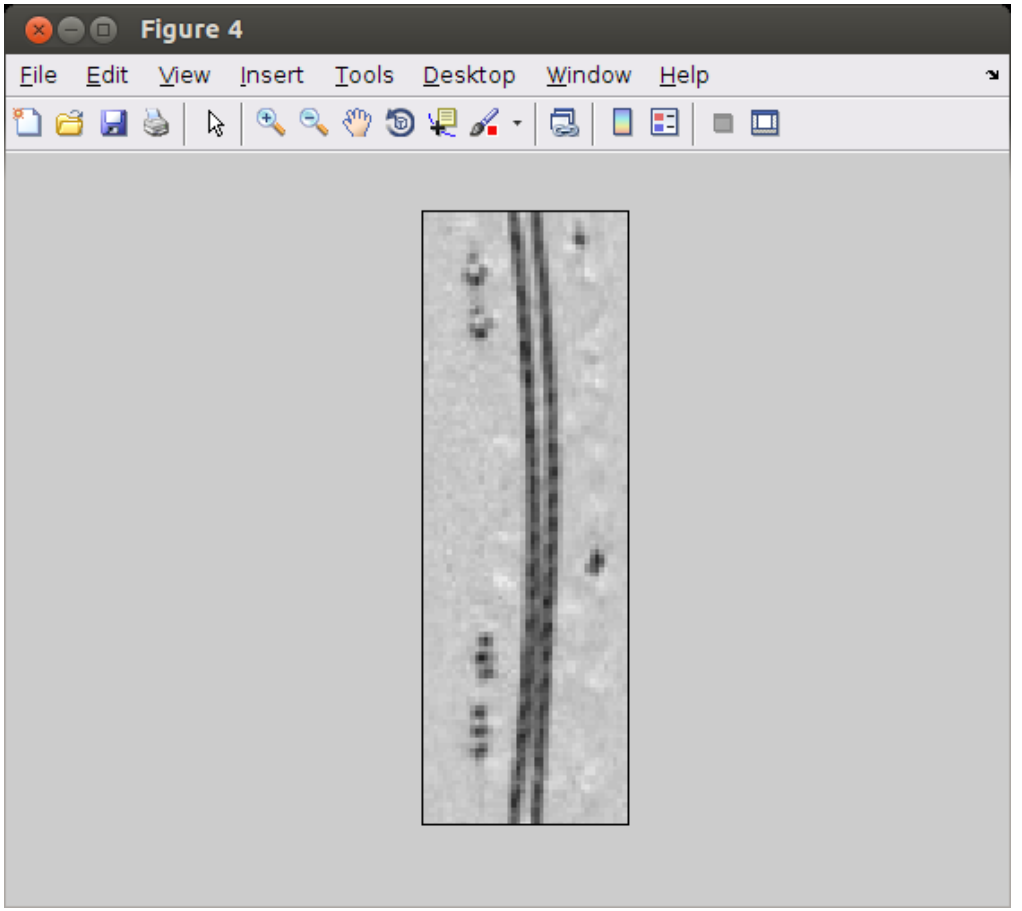
Some views will make the visual inspection difficult.



In such cases it is a good idea to activate echoing the image.



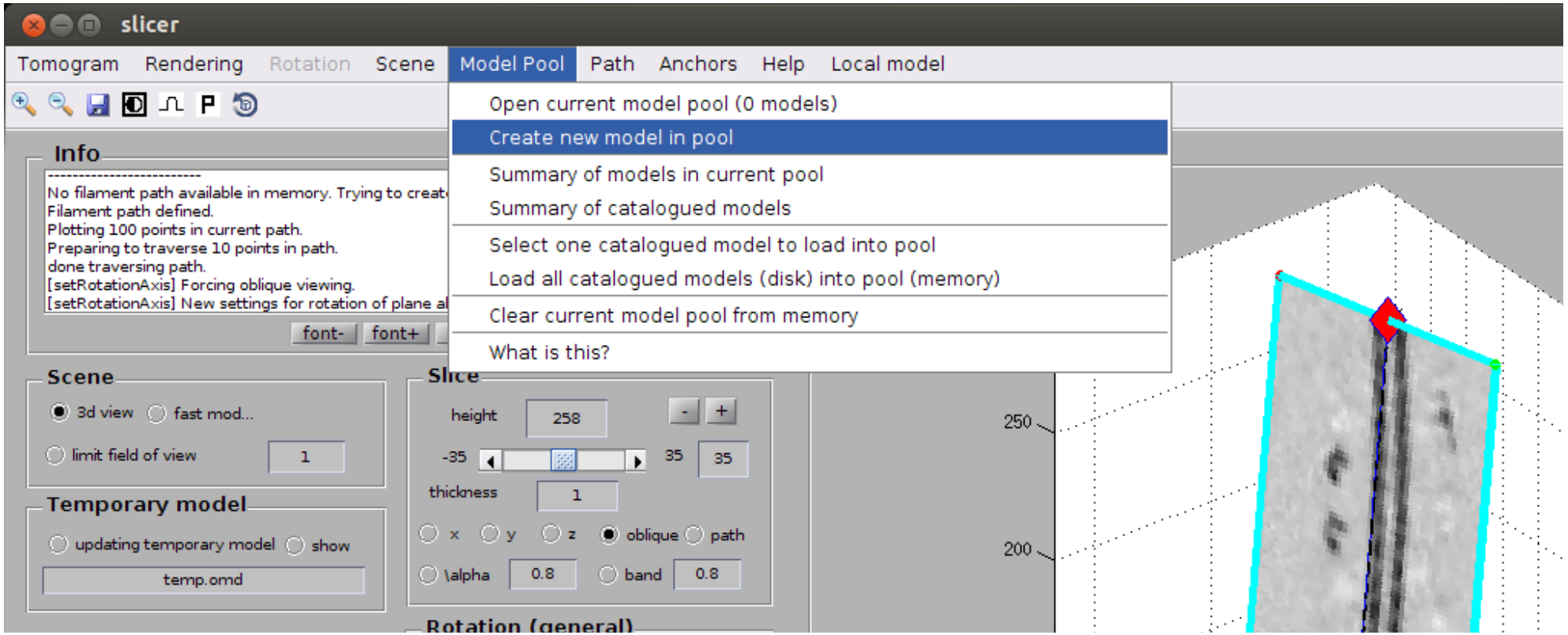
a better visualization can be attained
with the [echo external] option,
which echoes the depicted slice
in an external figure.



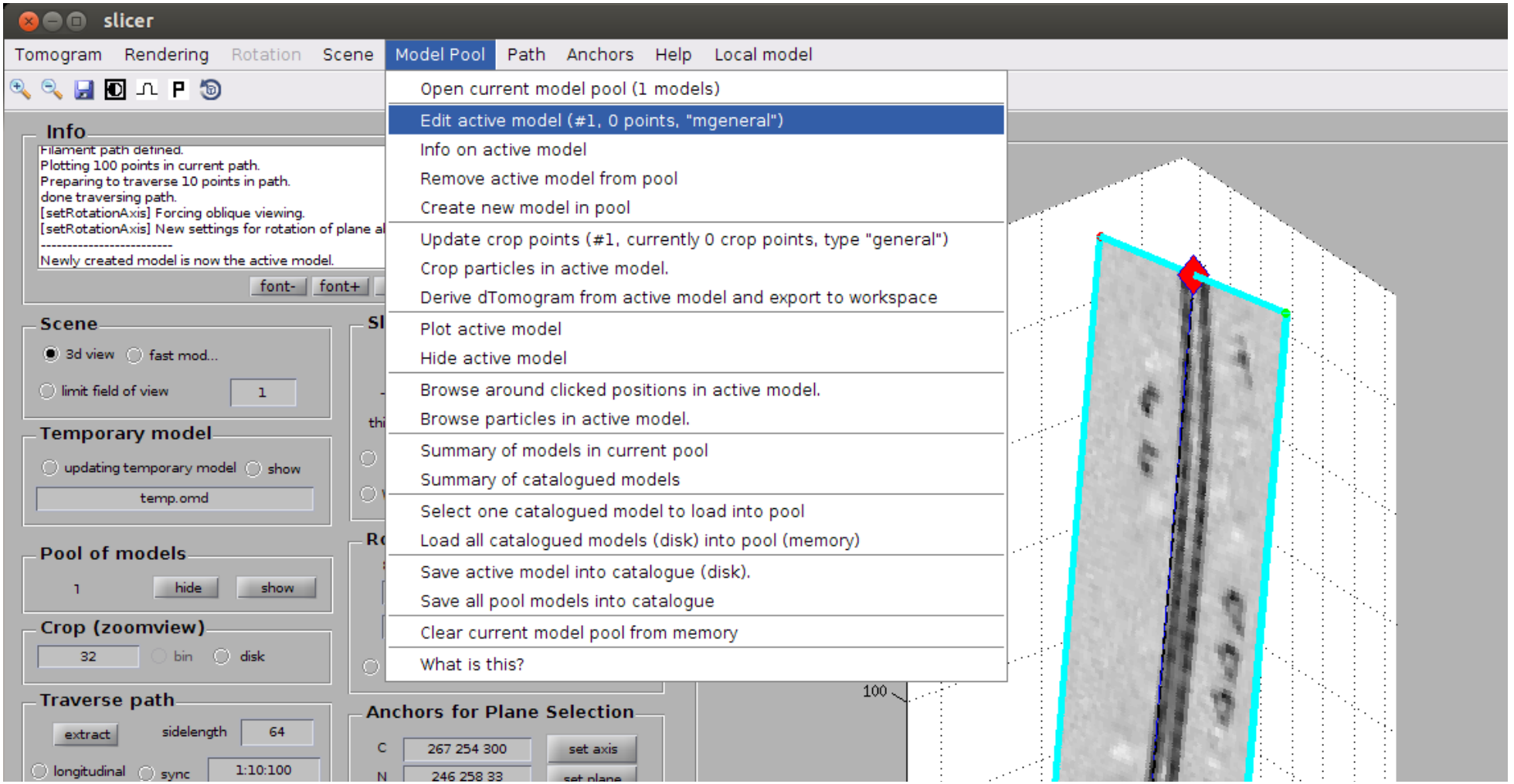
Now, we first define a model to represent a coarse approximation of the central pathway of the filament.

The “model pool” i.e., the set of models contained right now in memory referring to the tomogram under edition should be empty.

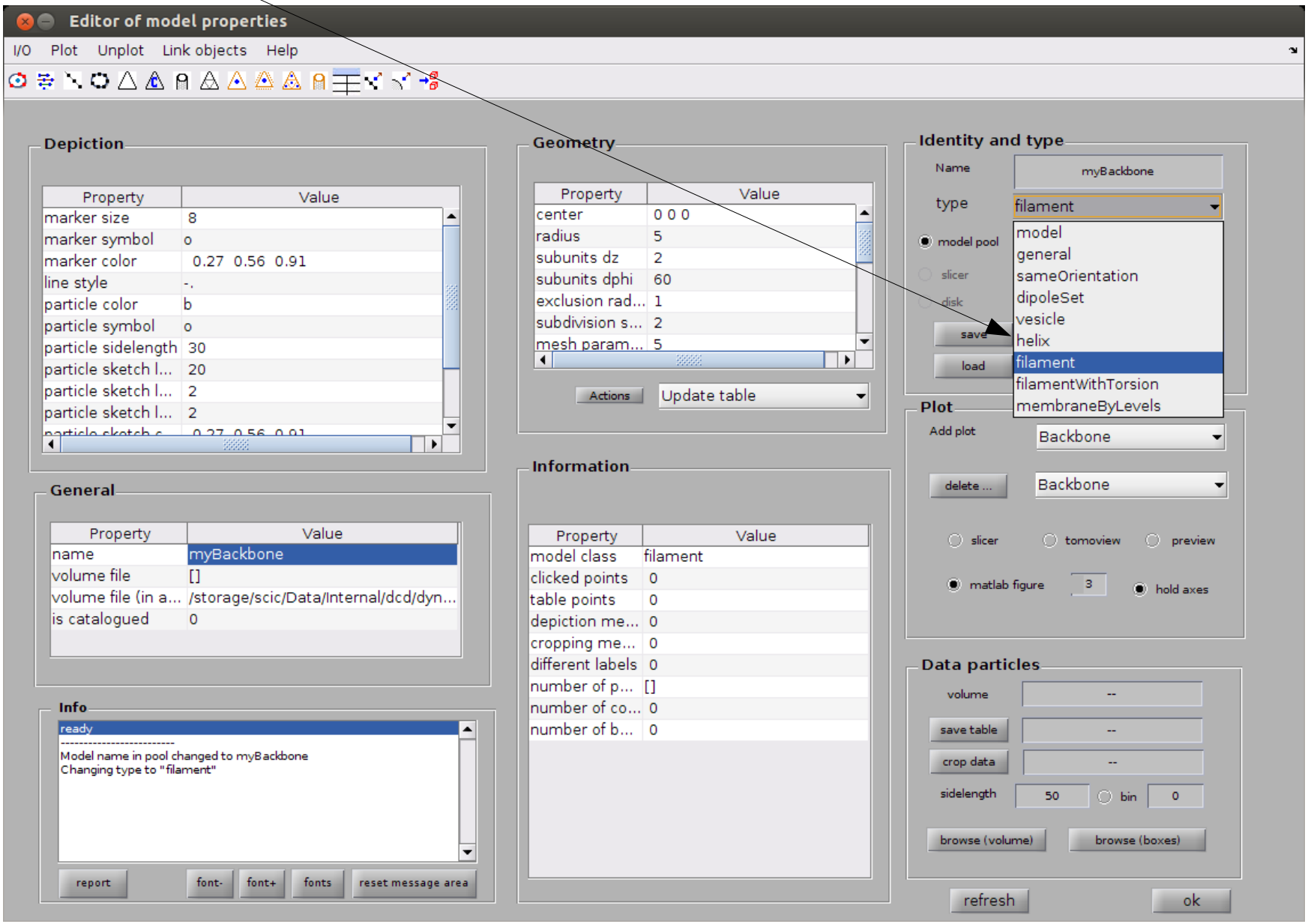
We just create a new model.



And then we can edit it to format it as the backbone of a filament

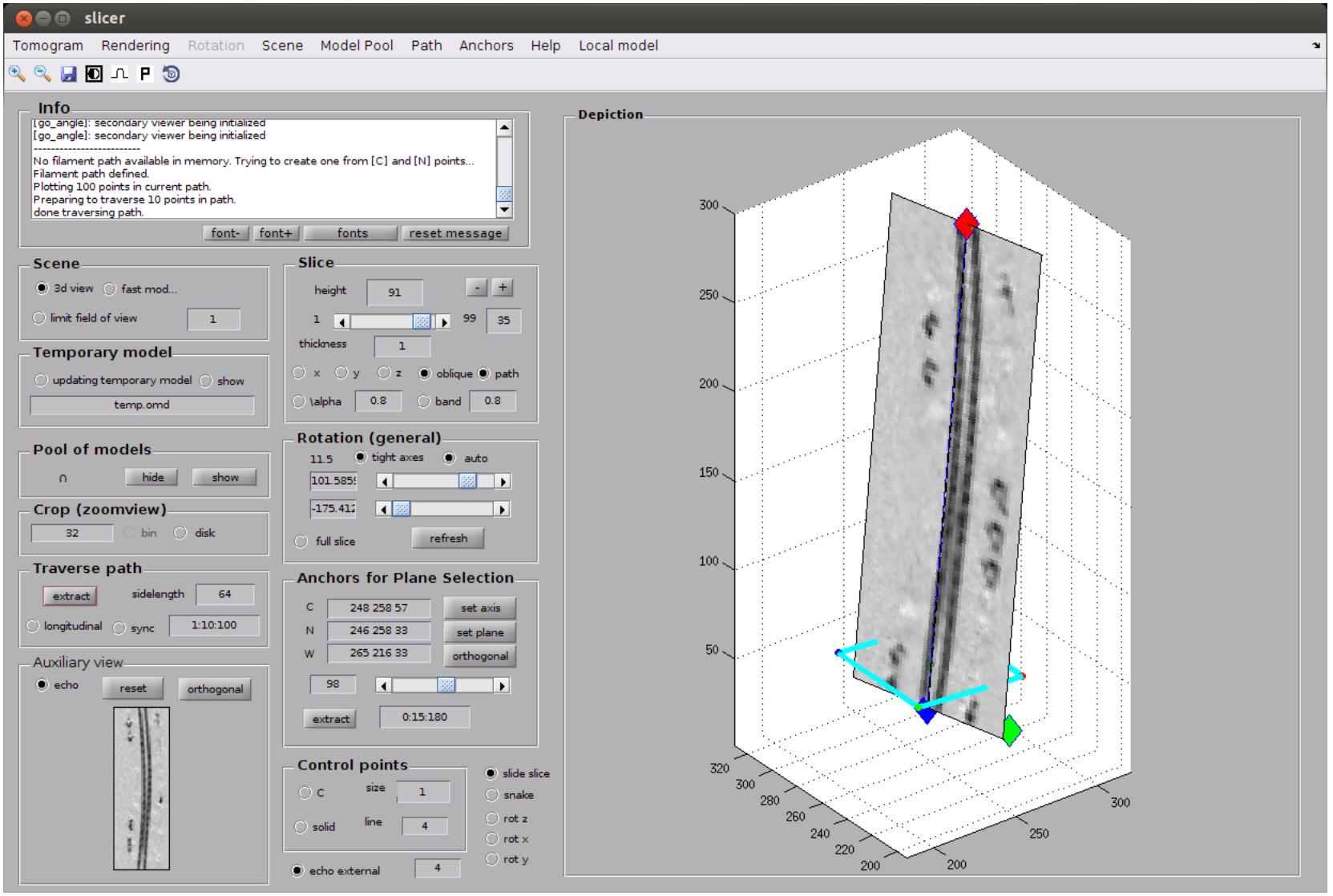


We select the option “filament”

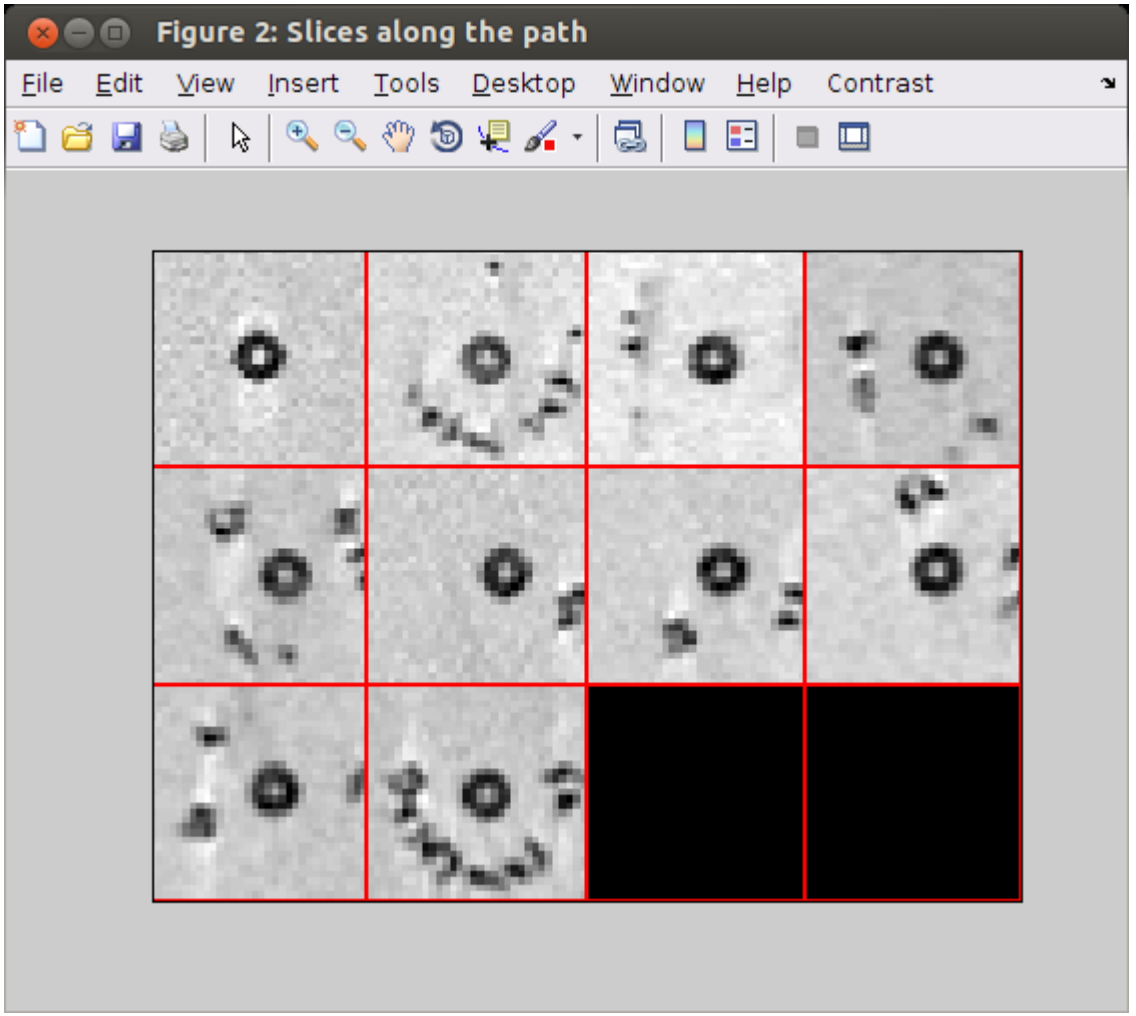


and it's also a good idea to change the default name to something more significative.
This name will also be used by the catalogue when you save this model.

The [C] (blue) and [N] (red) points induce a straight “path”.

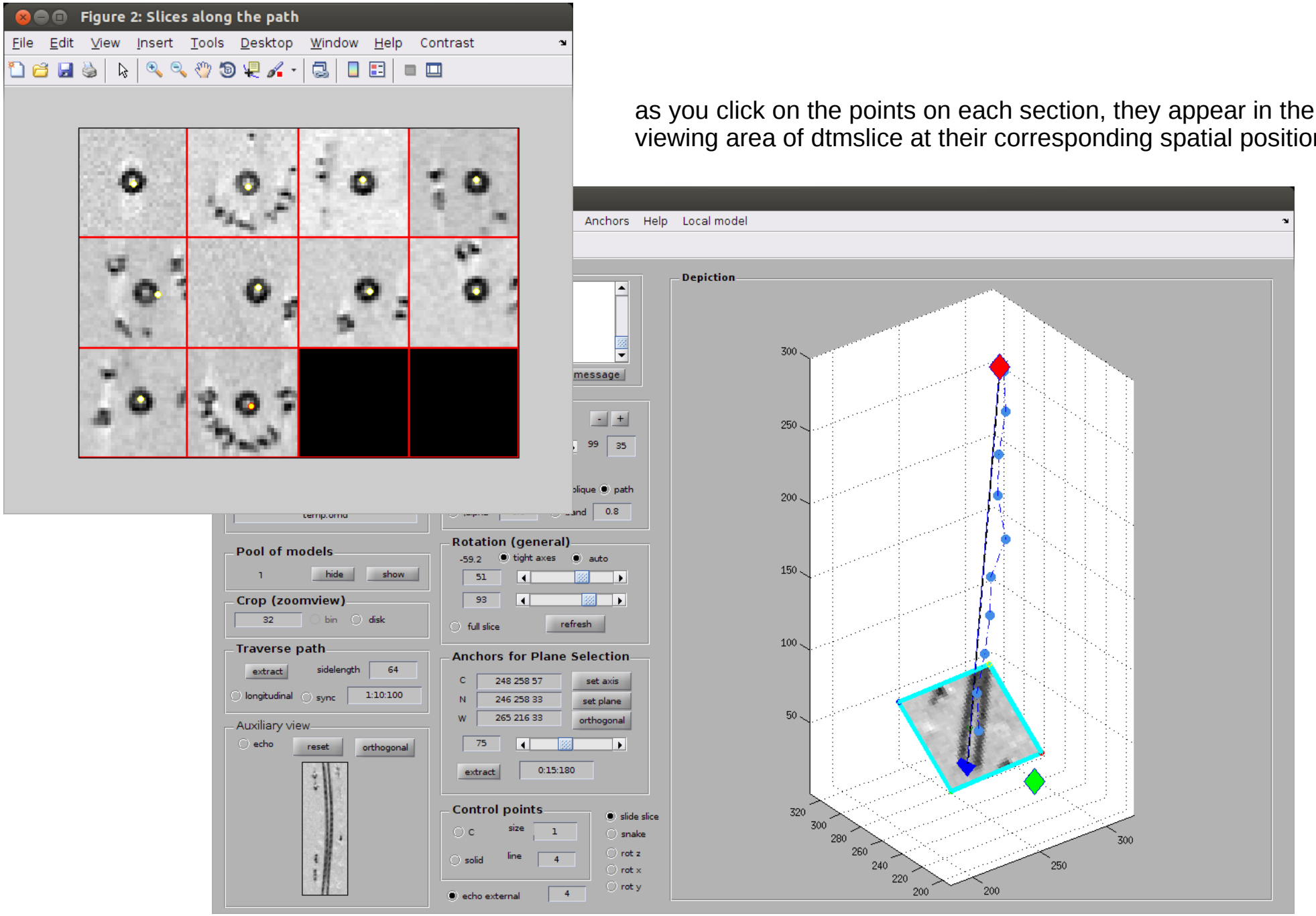


We extact now sections along that path. The centers of the filament on each section are now visible.



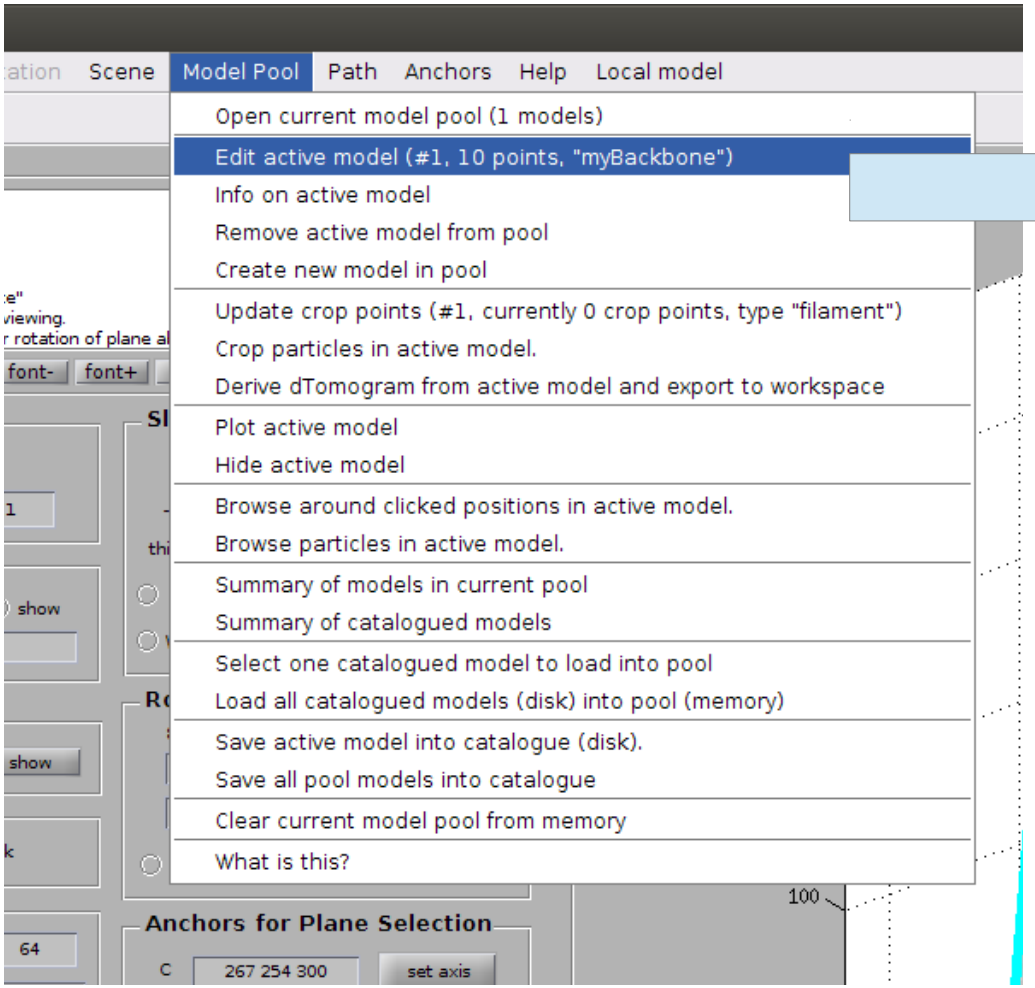
you can select the center of the filament section at each section pressing [c]
Delete with the [backspace] key.

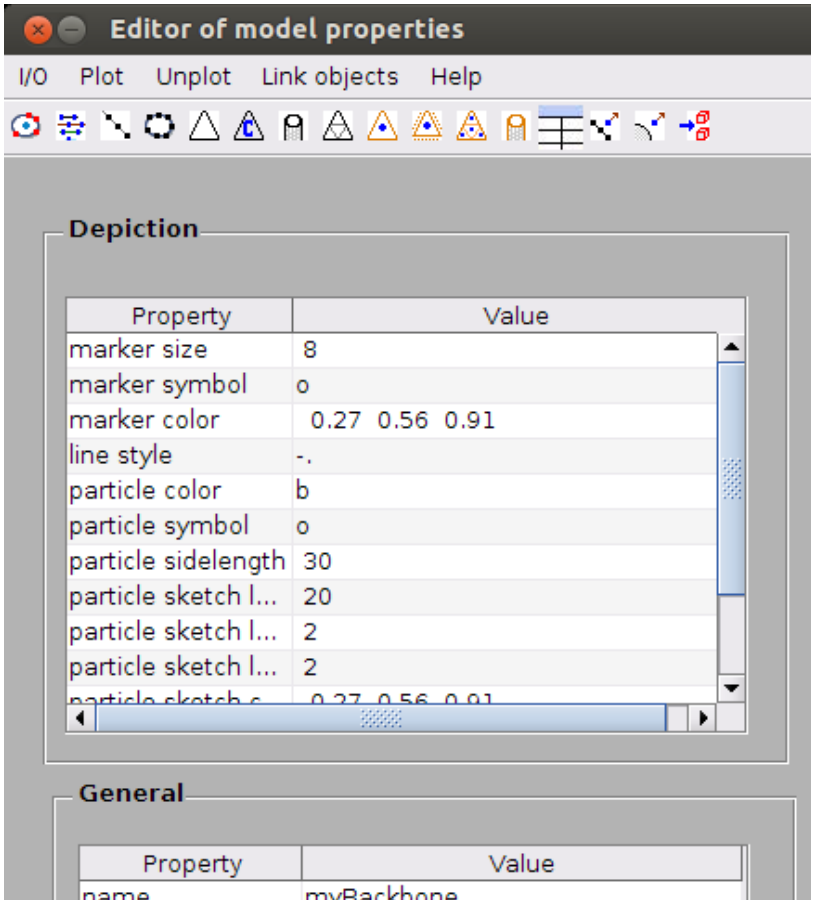
What you're clicking are the control points of a spline (a “backbone”) that will be computed later.



as you click on the points on each section, they appear in the viewing area of dtmslice at their corresponding spatial position.

Now that we have the controls points, we edit the model to create the “backbone”, i.e., the interpolant that traverses all control points.



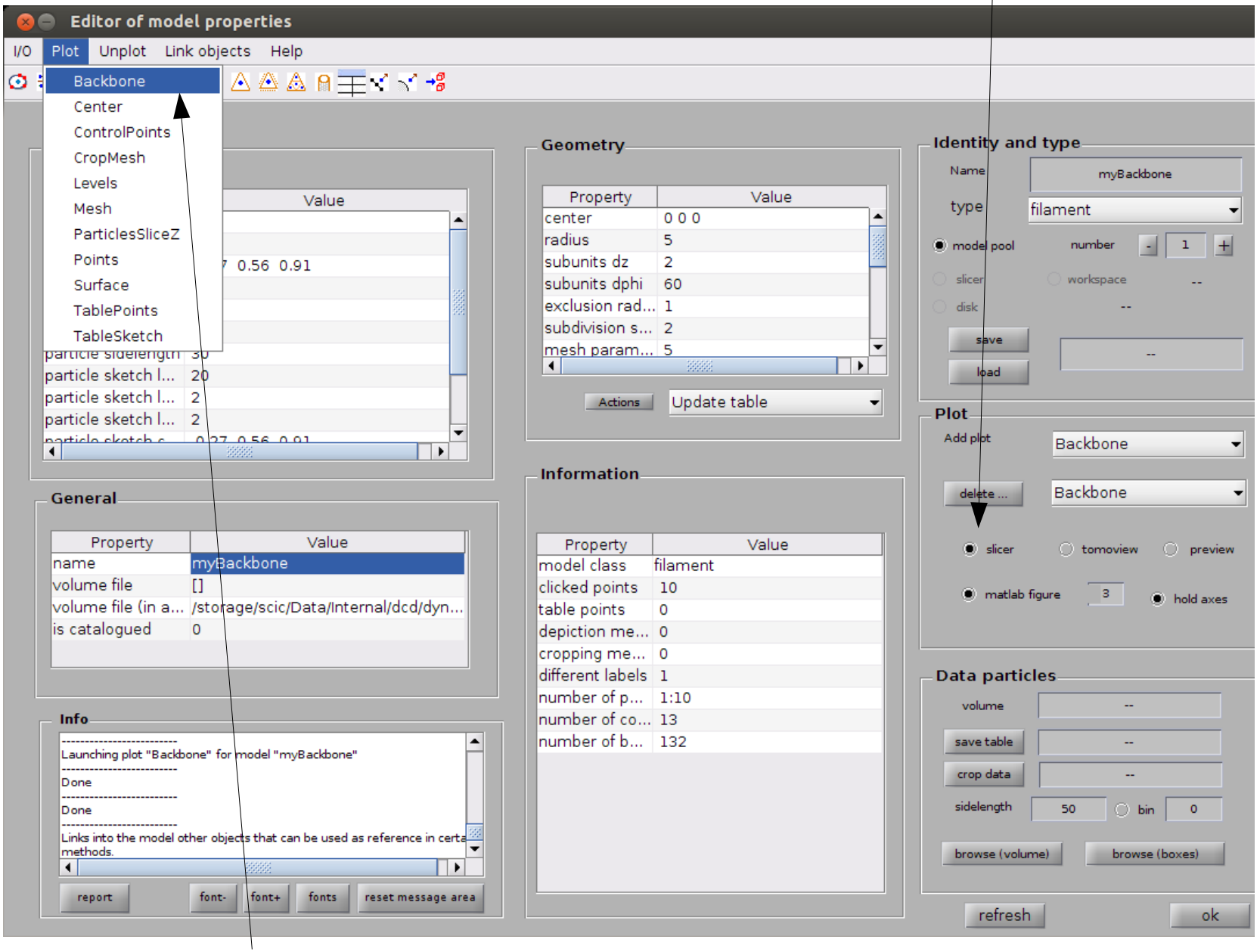


in `dslicer`

in `model_edit`

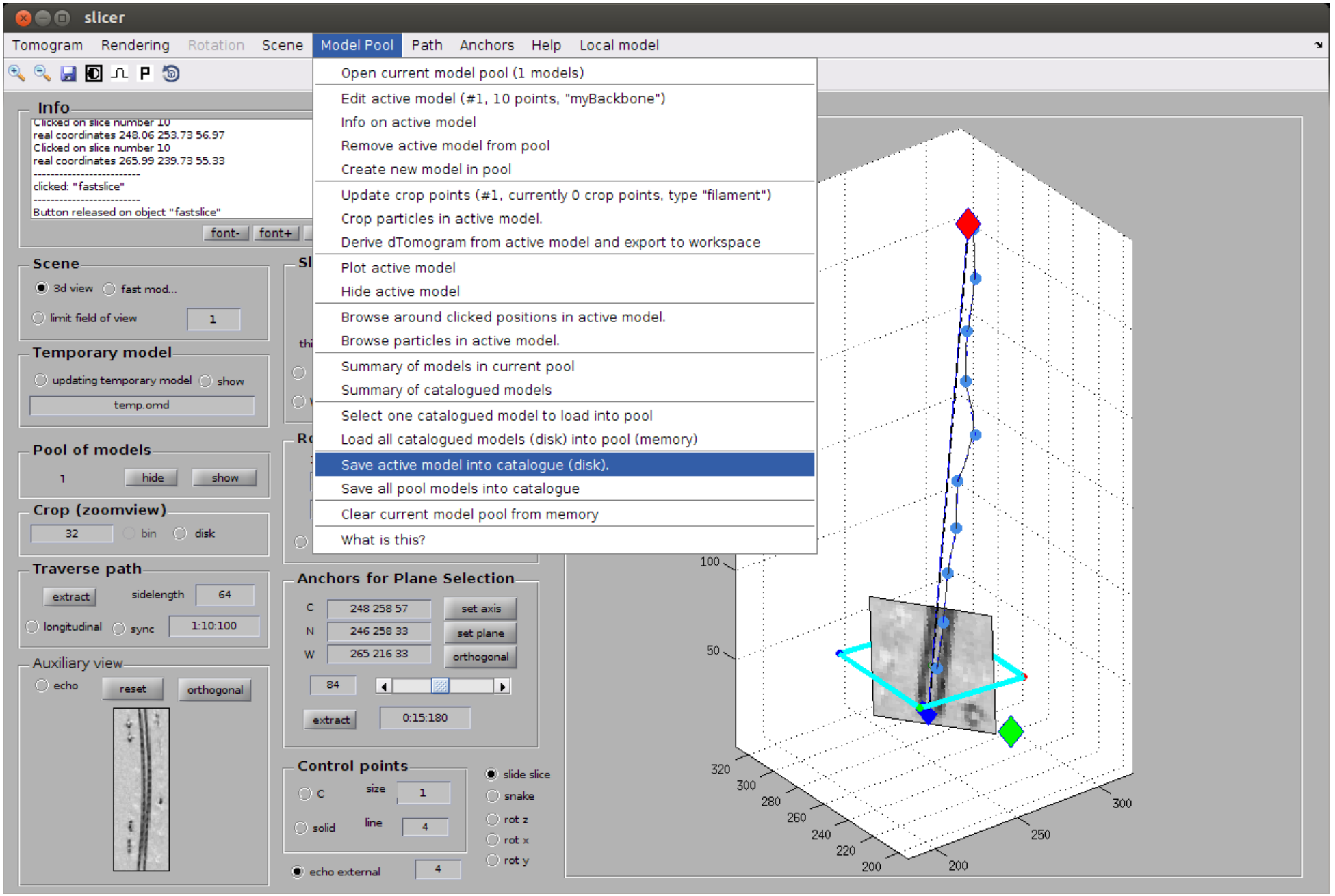
we can check graphically how the backbone looks like.

we first inform that we want a graphical depcition in the slicer window



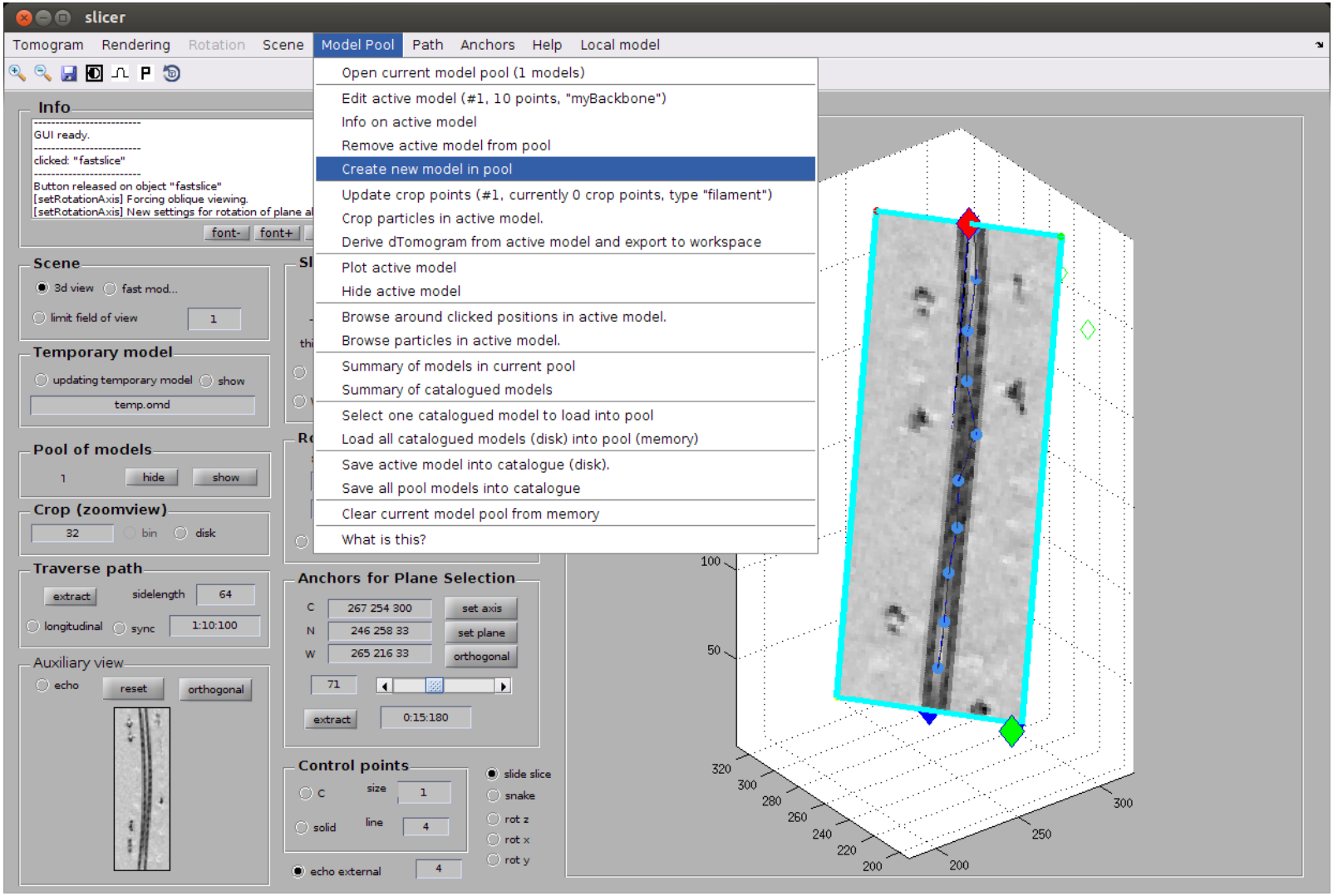
and then we order a plot of the backbone

as it looks good, we just save it to disk using the catalogue link



This first filament model is intended to be used as reference for orientation of the particles that “float” around the filament, as we assume that they are approximately equally oriented with relation to the filament.

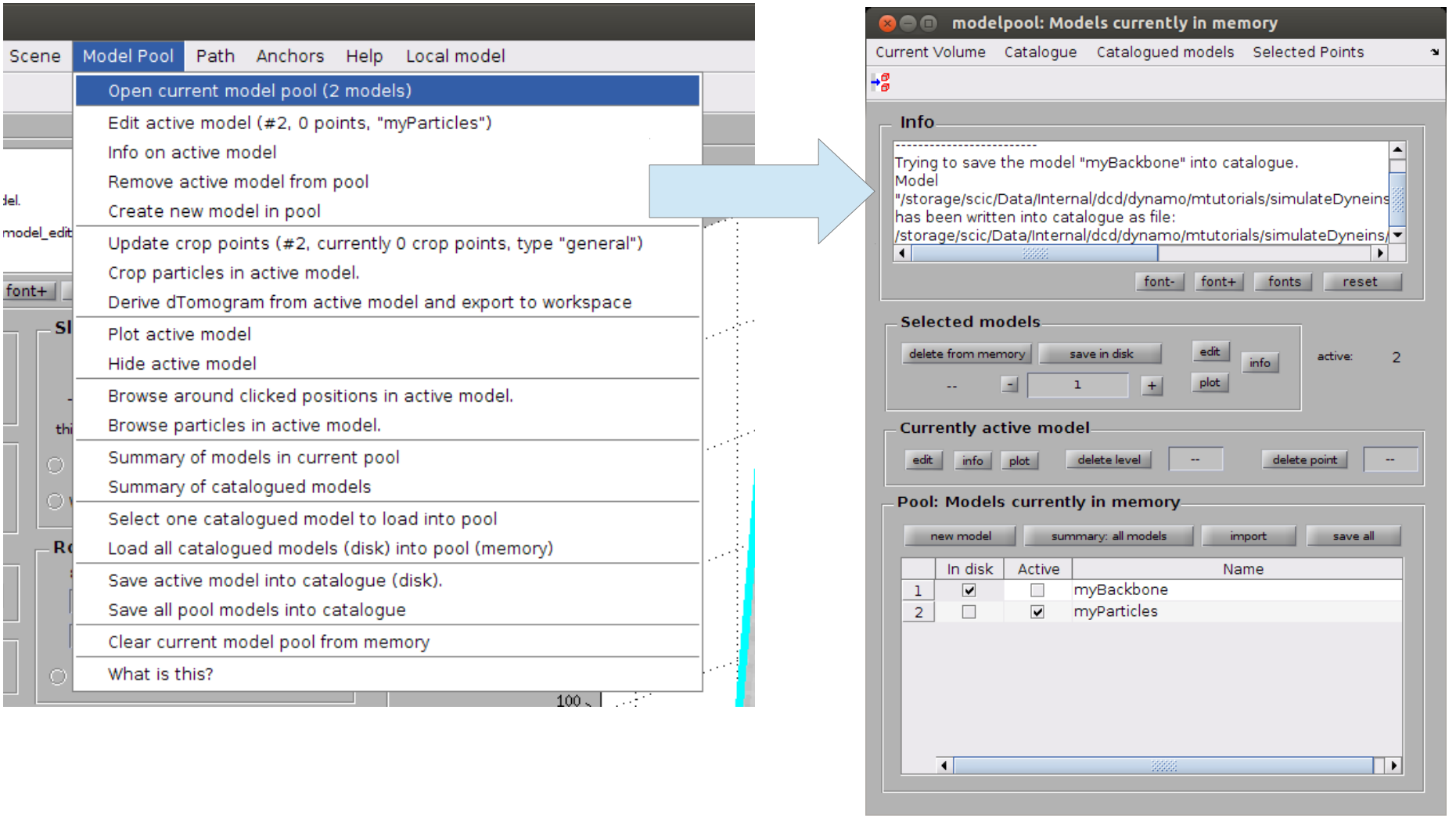
Thus, we need to define a new model, where we will click these particles manually.
and afterwards the previous “myBackbone“ model will be linked.



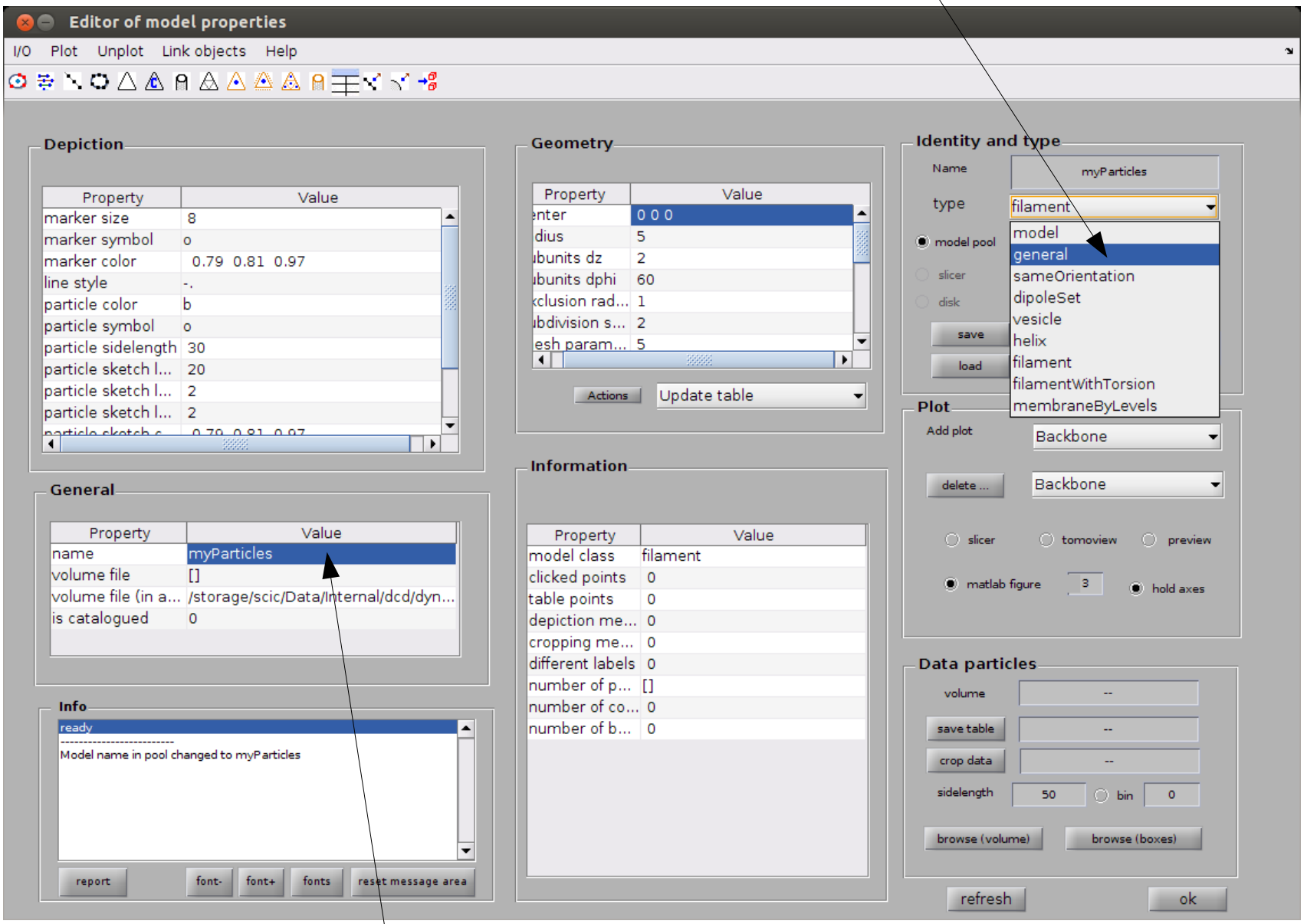
The newly created model becomes autamtically “active”, meaning that all your intervention with Dynamo from this point on will be referred to this model.

The points you click will be included into this model.

You can check which model is active in the modelpool menu:

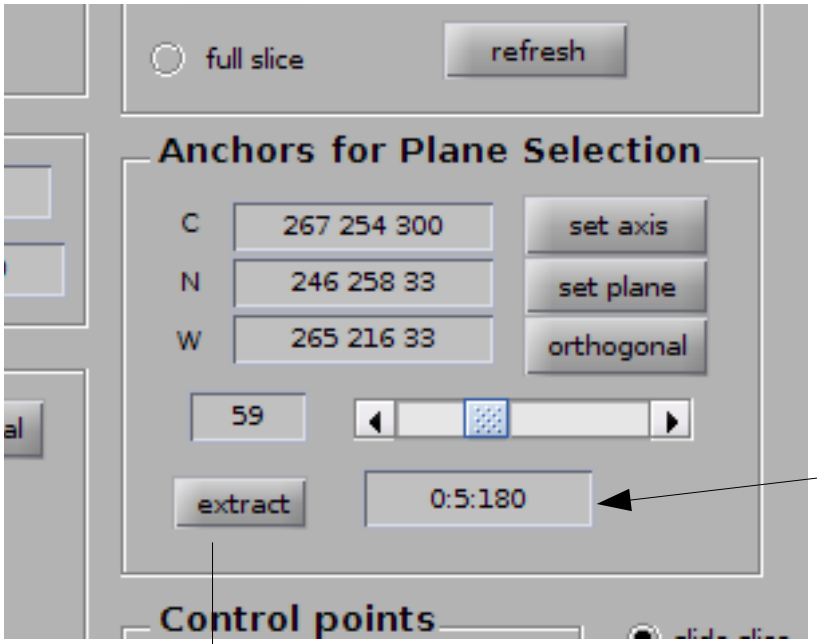


In any case, we need to edit this model [Model Pool > Edit active model] to make it of type "`general`". By default, it was created as "`filament`" (the type of the previous model)



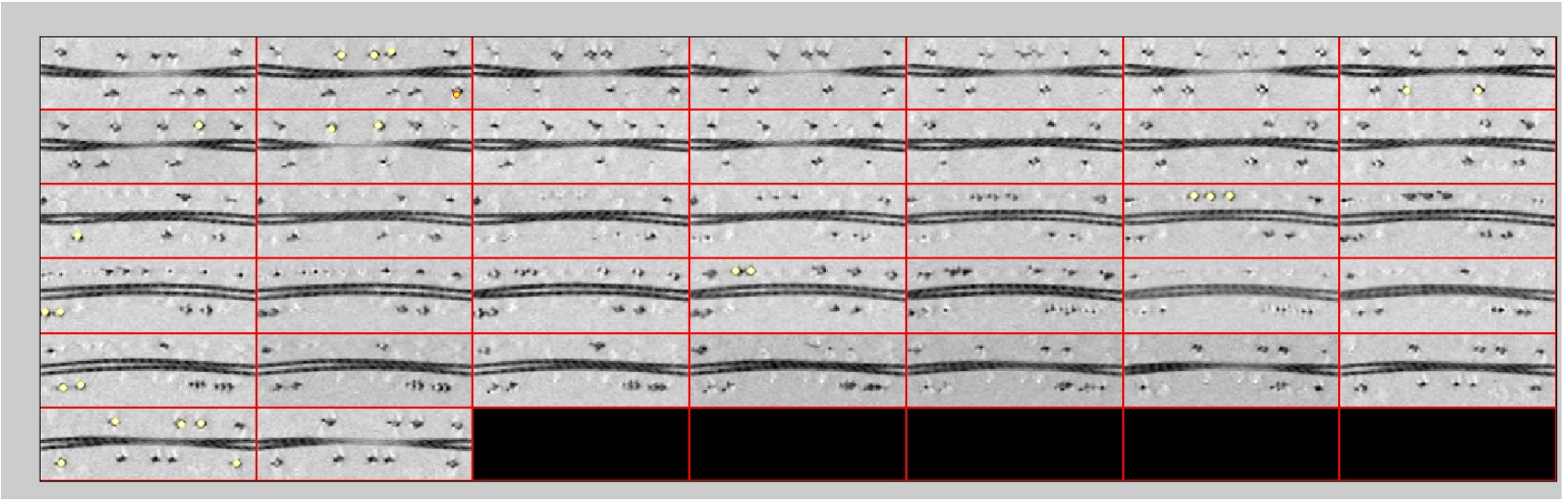
Also, it is a good idea to change the name of the model, for instance to "`myParticles`"

Picking manually the particles is easier visualizing them in relation to the filament.
The adviced procedure is to create a set of planes that rotate along the straight path that models the filament axis.



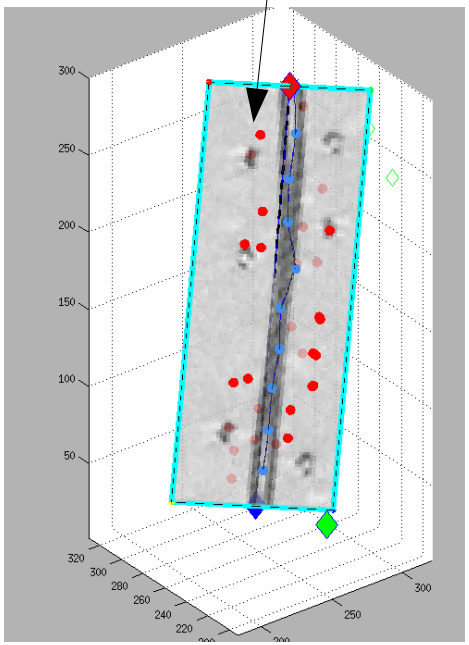
creates planes separated 5 degrees from each other

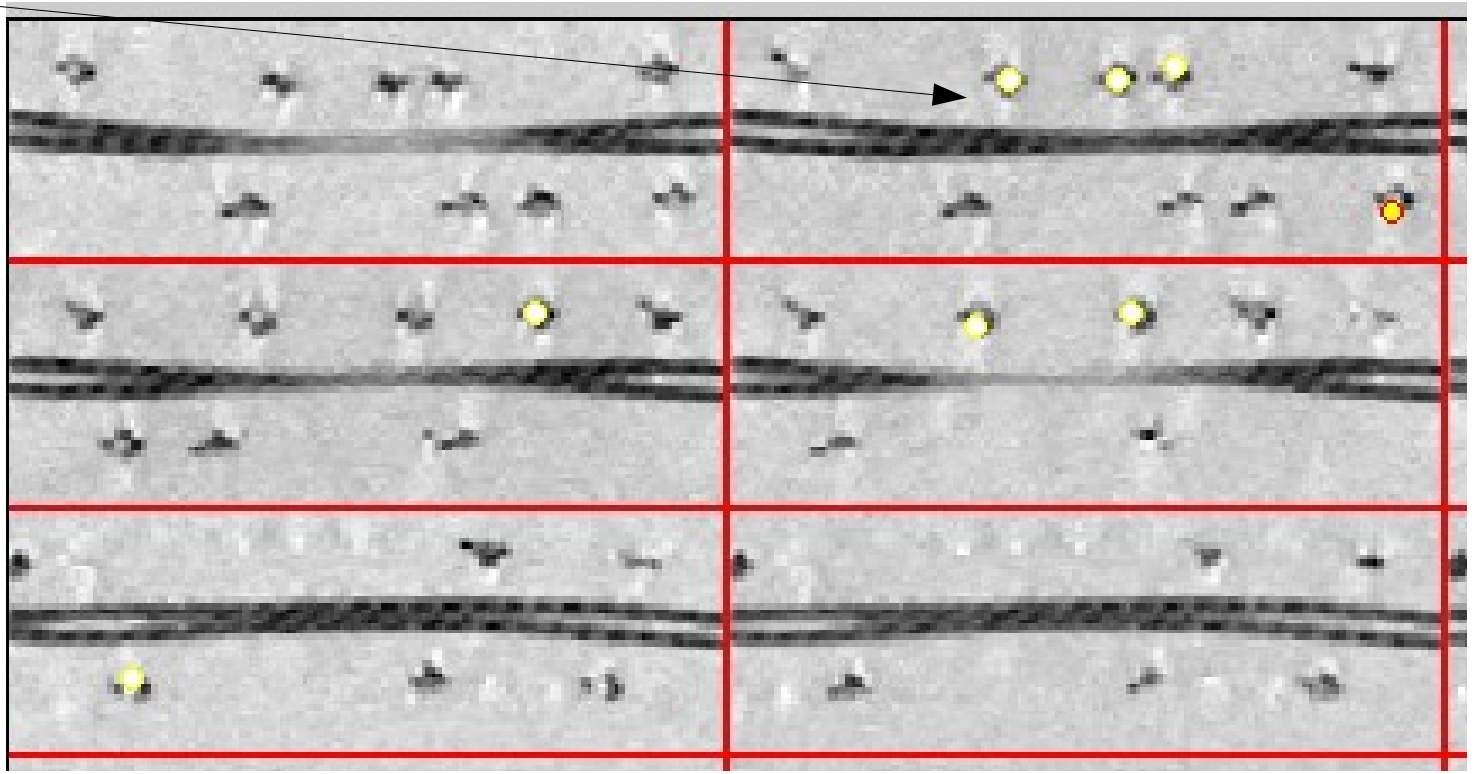
Press to generate the figure



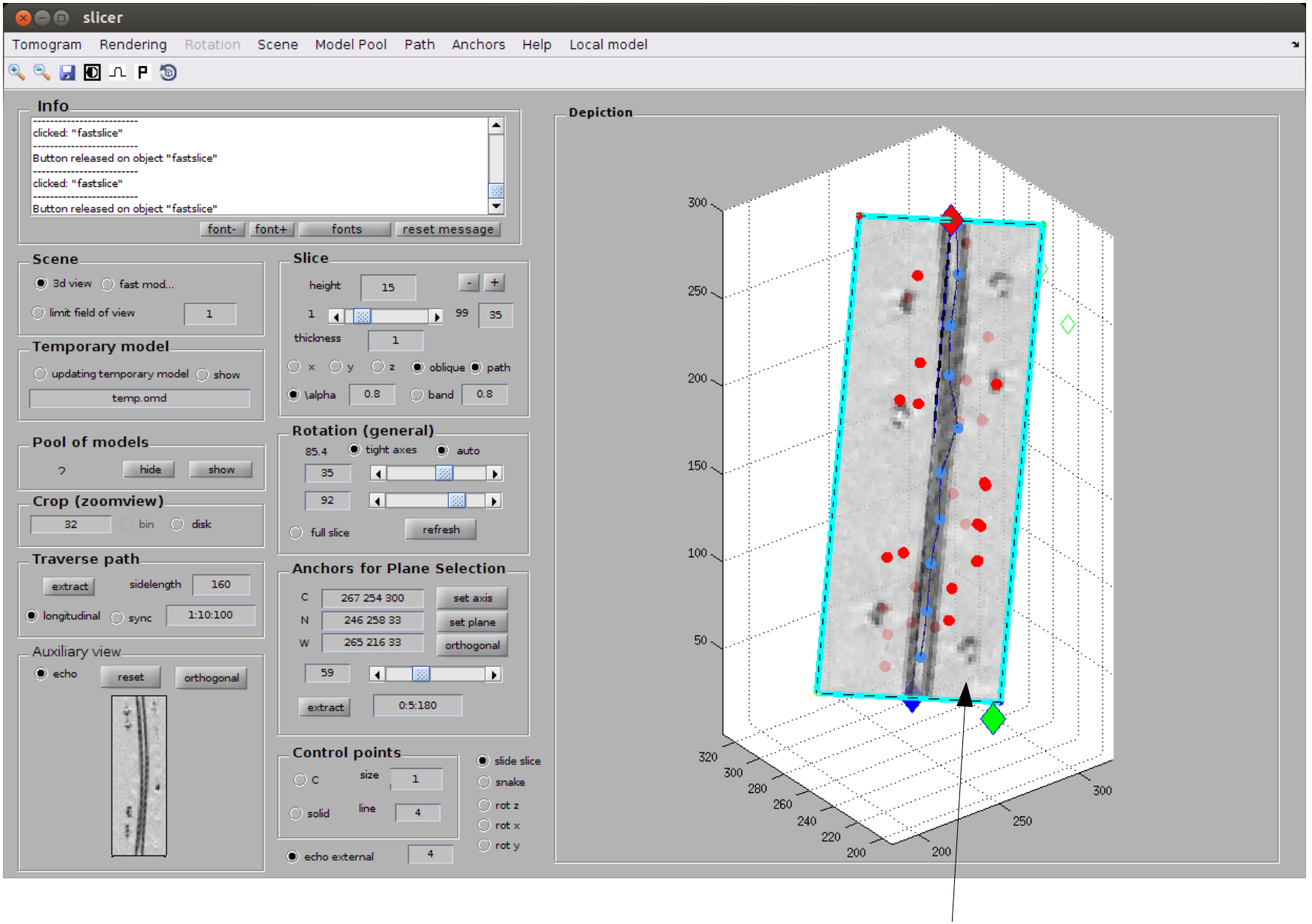
You get a montage of all slices traversing the axis (with an angular interval of 5deg)

.You can click points on them
using [c].
The points get echoed in `slicer`

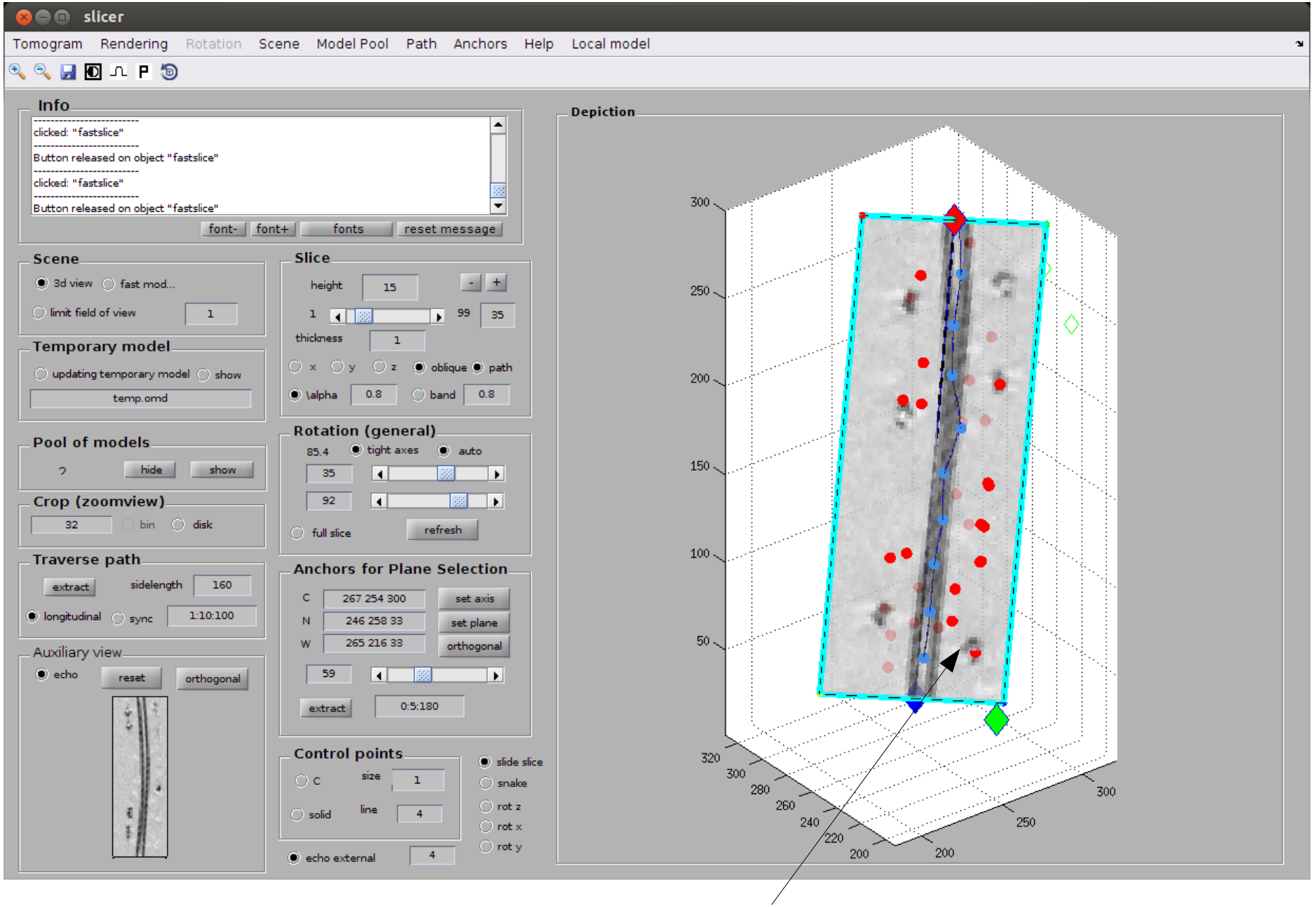




These points appear on the screen. You can rotate the slice to compare coincidence of points and density map.

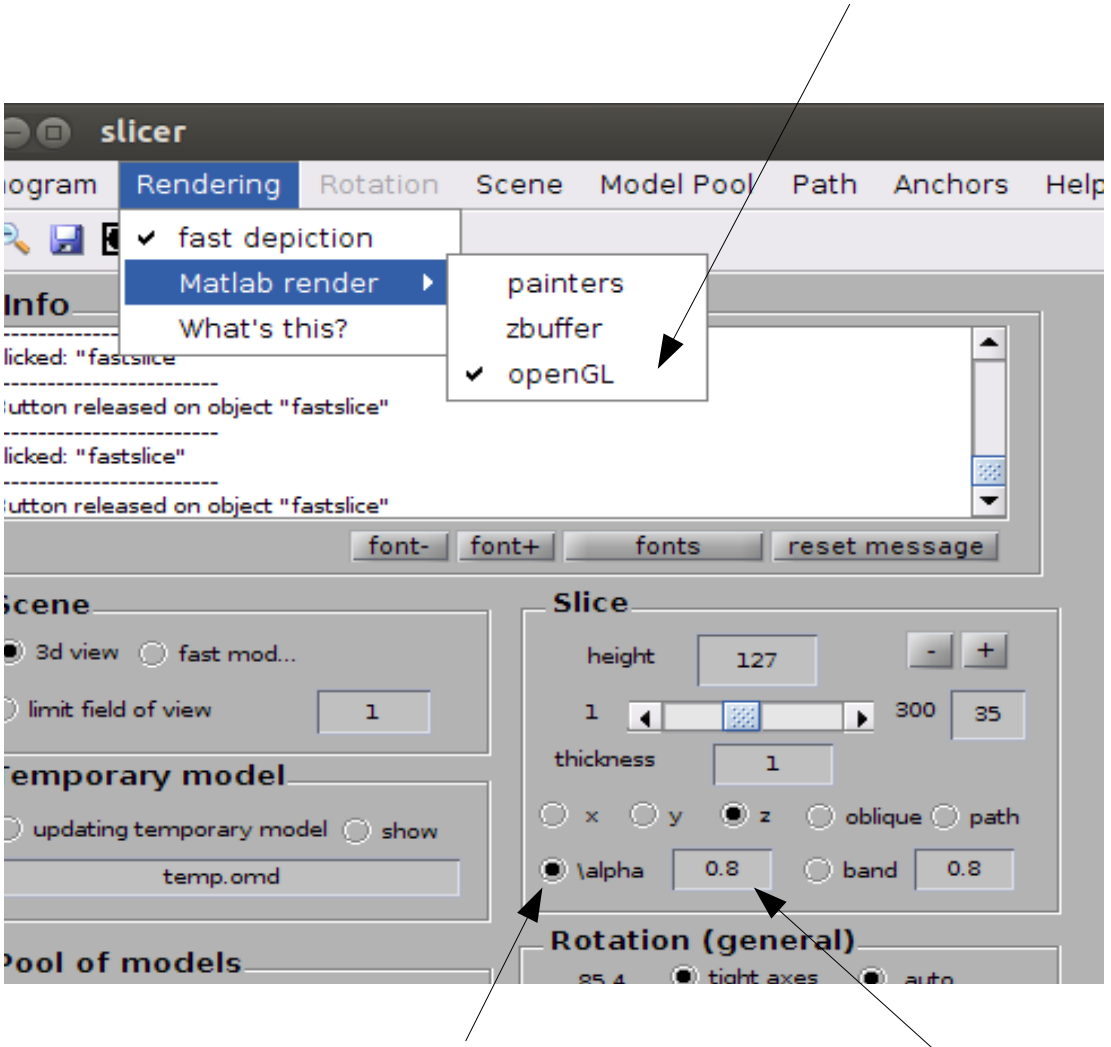


… and localize visually the spots that you have missed on the first pass (intensities without a color)



which you can click directly in slicer (click [c] on the spot in the slice)

For this visual check, you may need to enable the transparent rendering of surfaces.

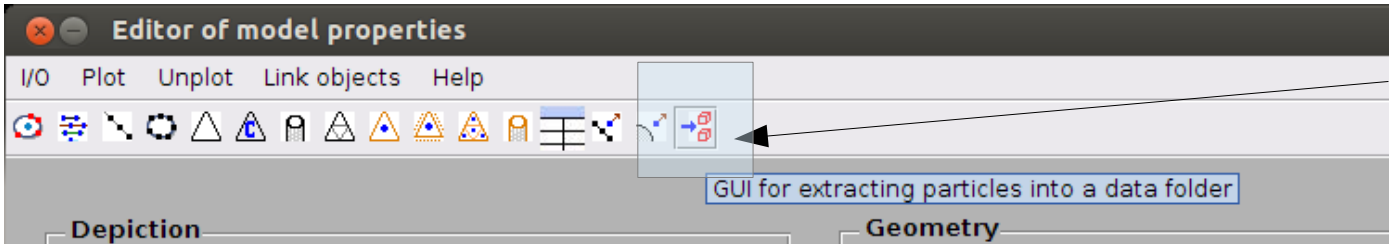


activate transparency level

choose trnasparency level

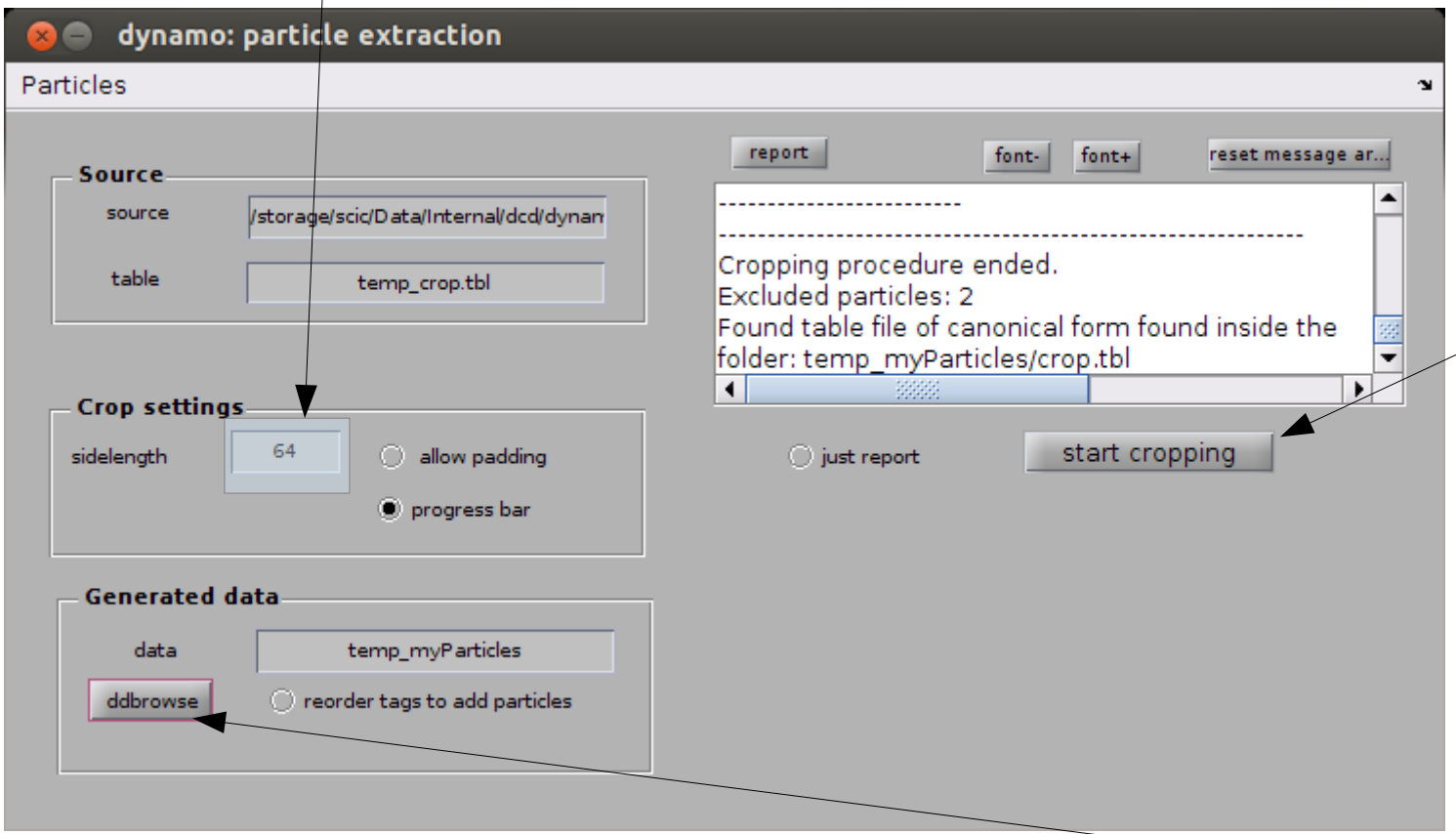
What happens if we try to use the particles in this model?

We could just crop them and inspect them:



In the model editor, we click the icon for particle extraction.

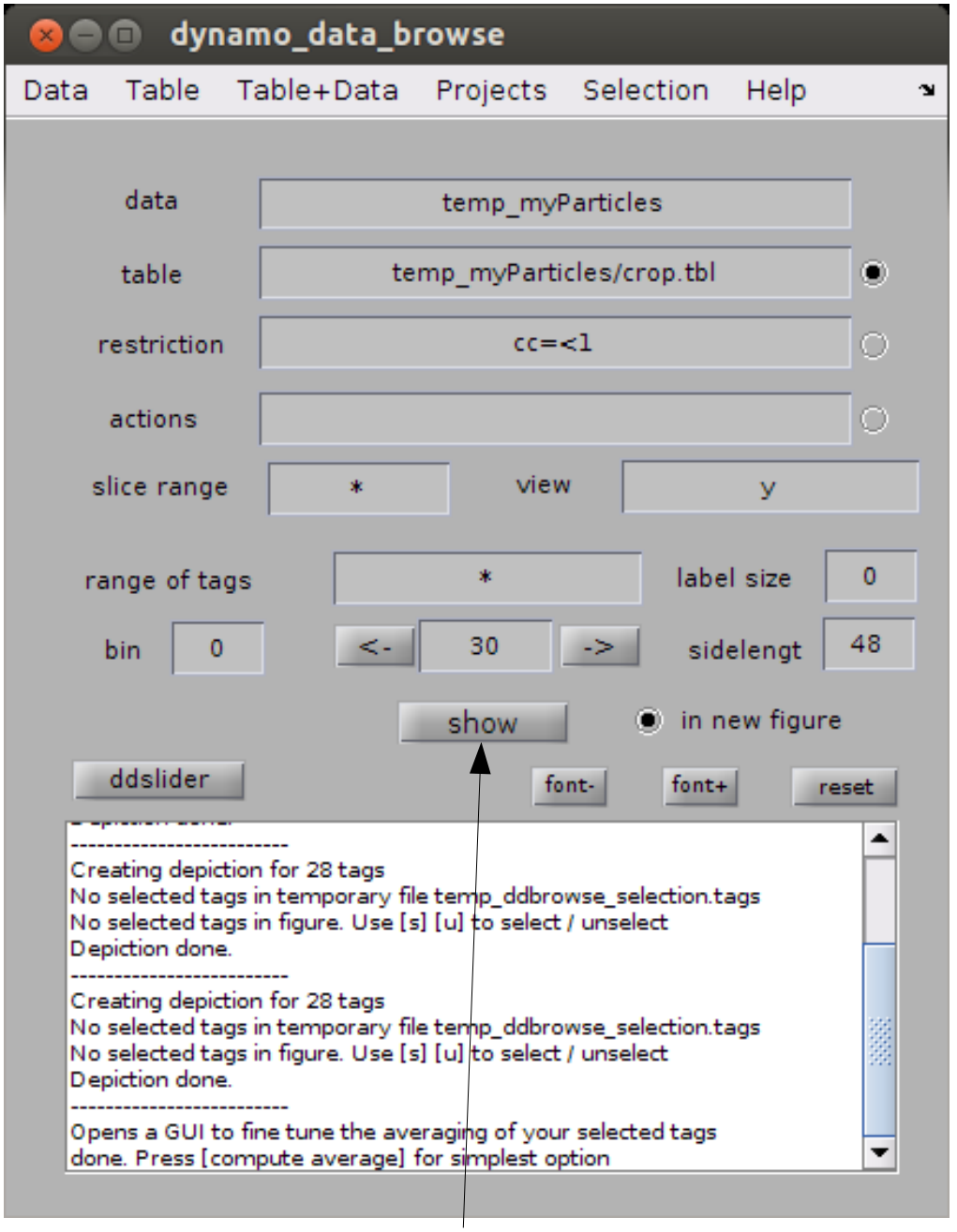
we select a sidelength for cropping each particle



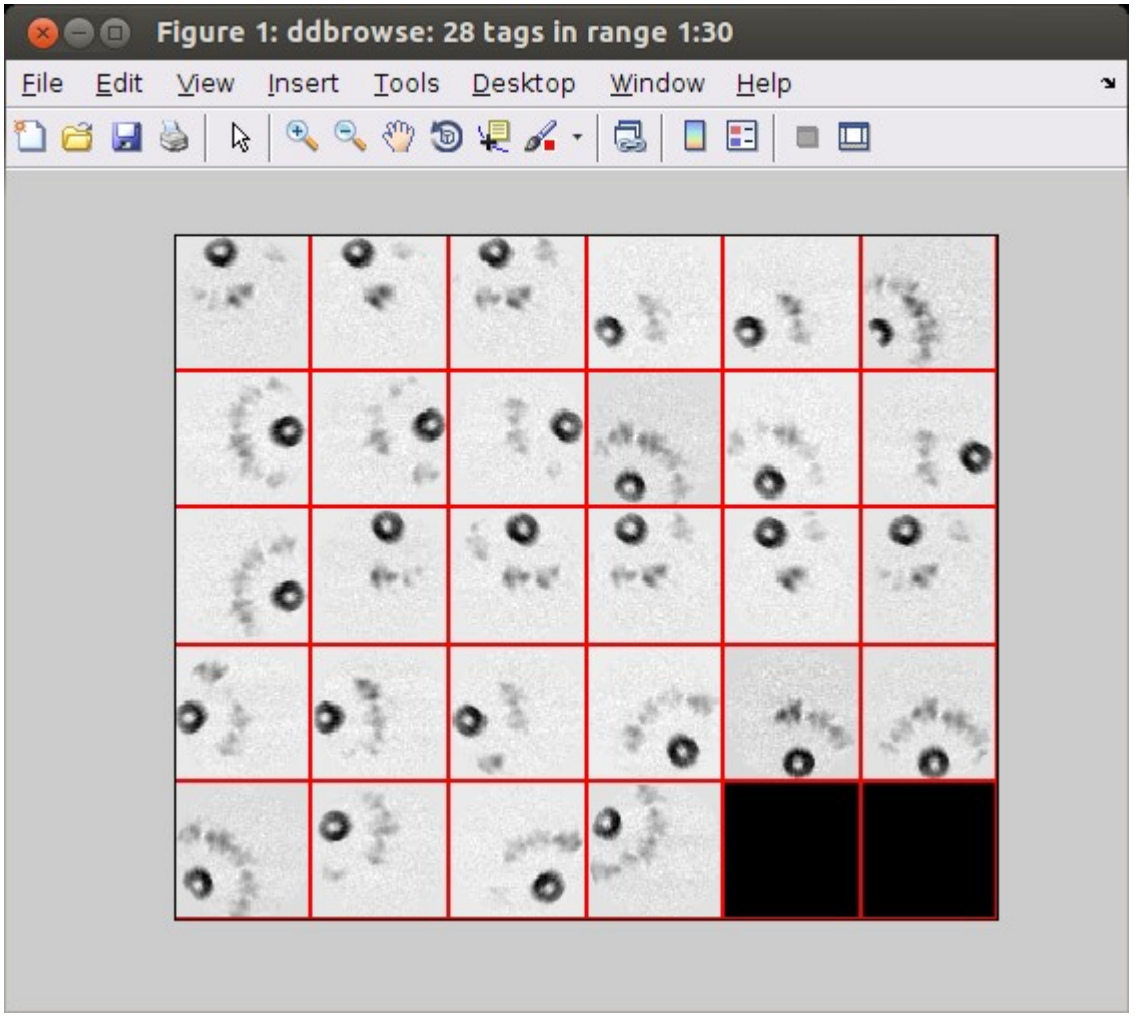
start croppping

this automatically produces a *Dynamo*-formatted data folder and a *Dynamo* table (with metadata).

the resulting data folder and table can be directly inspected with `ddbrowse`



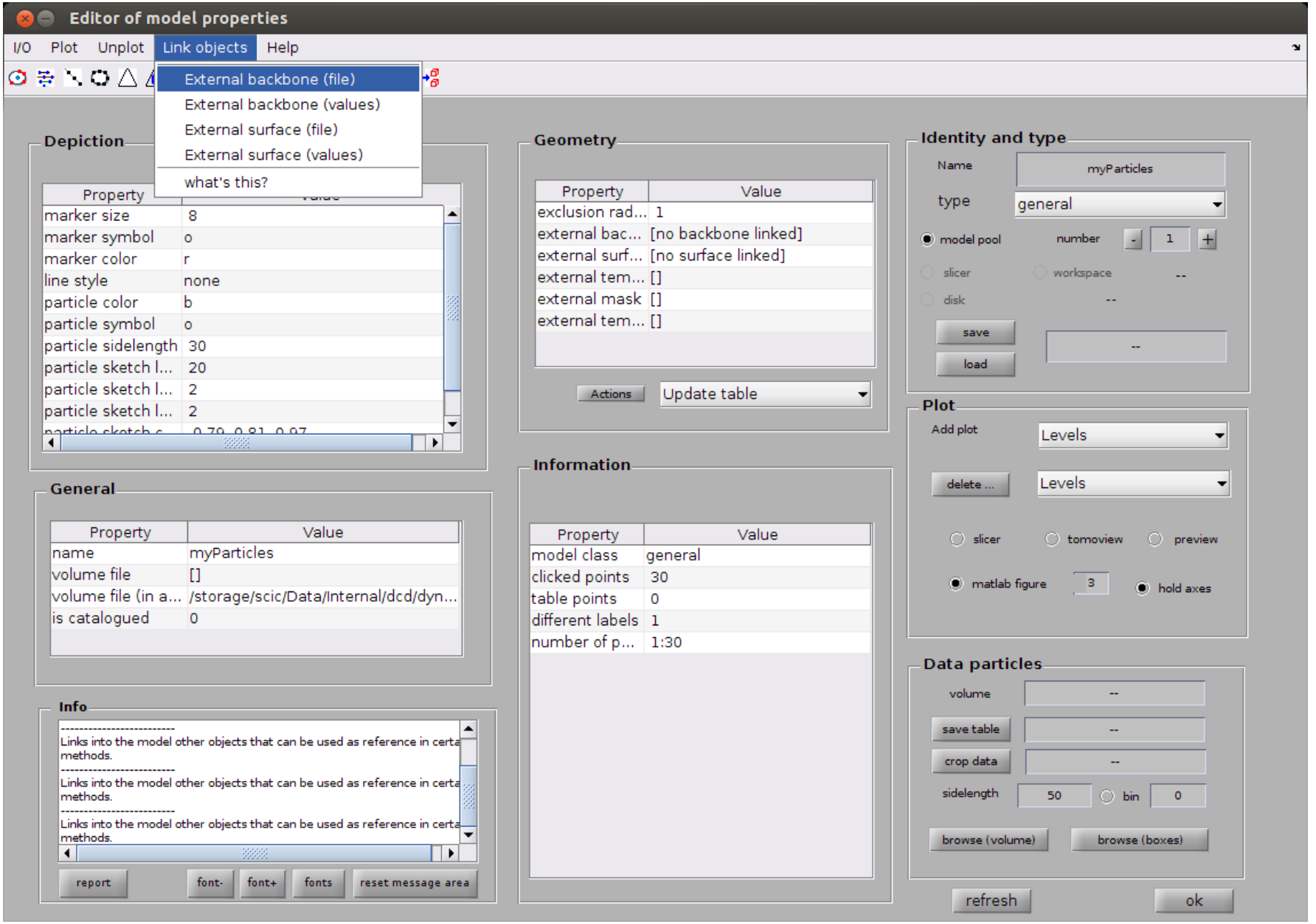
… when we visualize the particles....



we see that they are still oriented as they are in the tomogram.

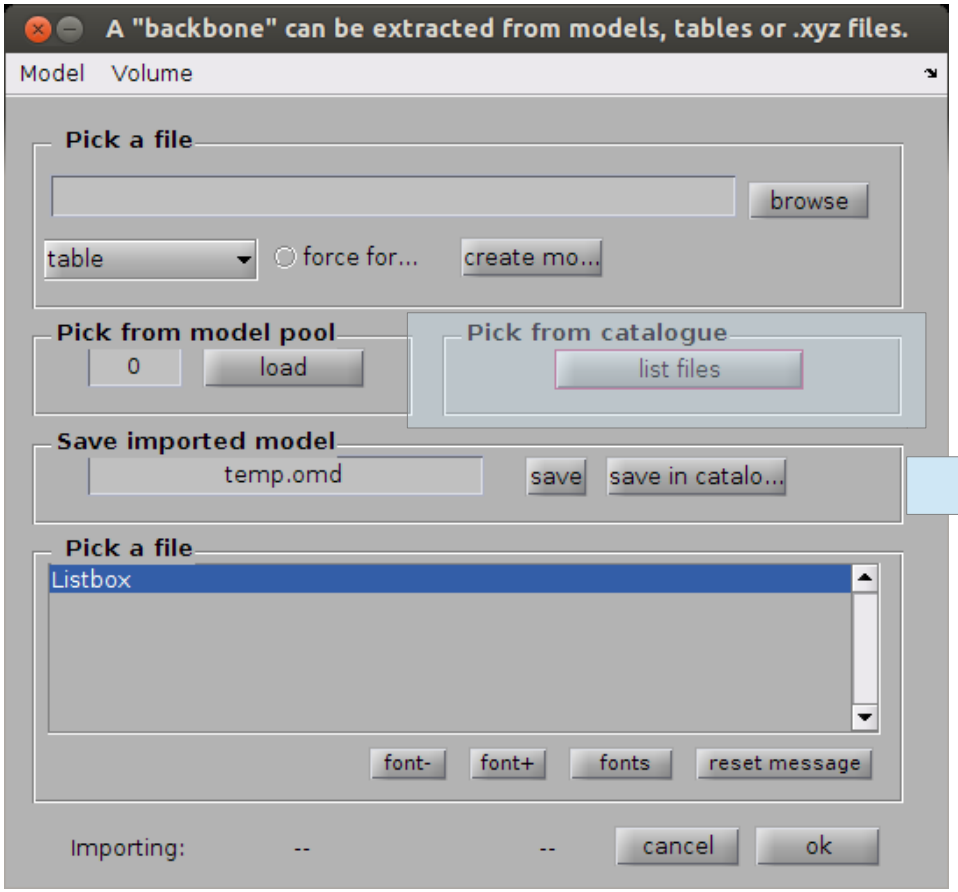
We haven't linked the clicked particles with the reference filament yet.

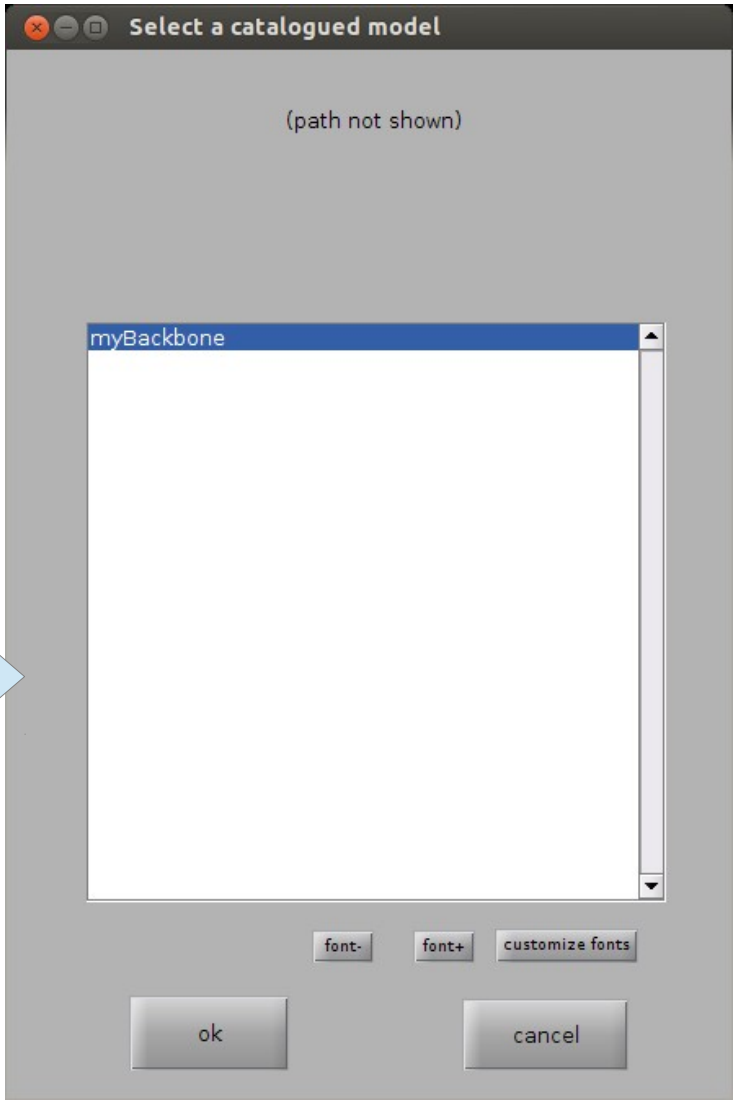
so, we tell the model edito that we want to link a backbone (that we have in a file)



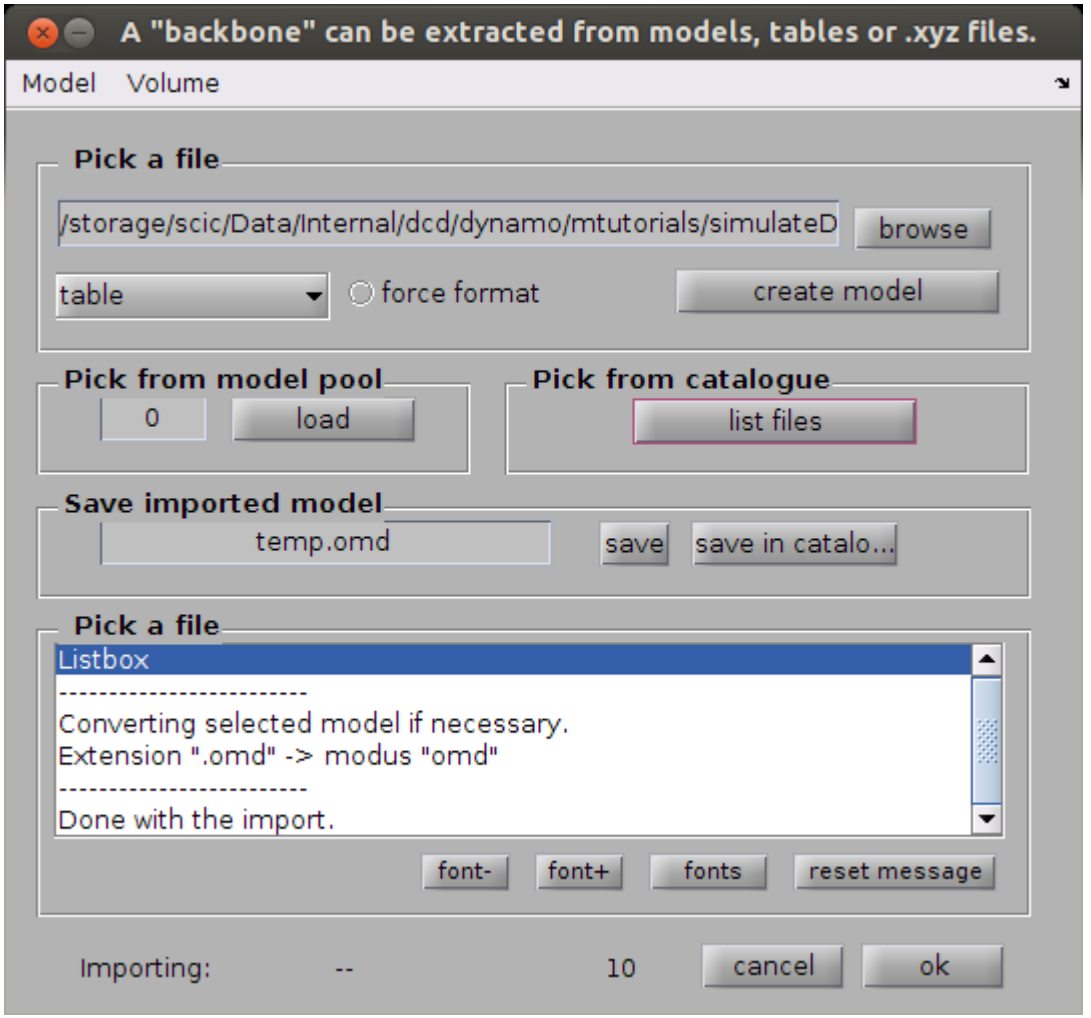
we have saved our backbone file in the catalogue, so it should be there.

press on [list files]





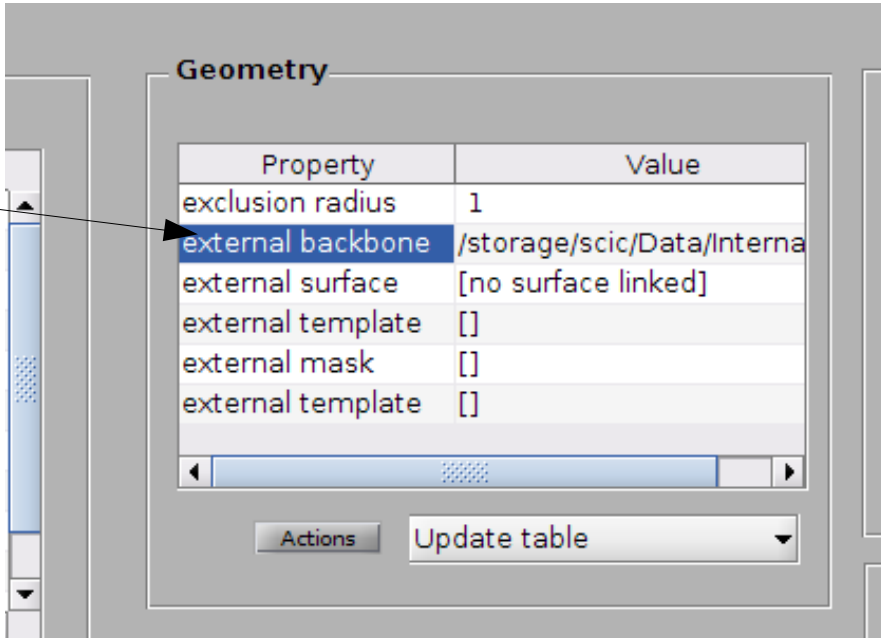
bingo! select it and click [ok]



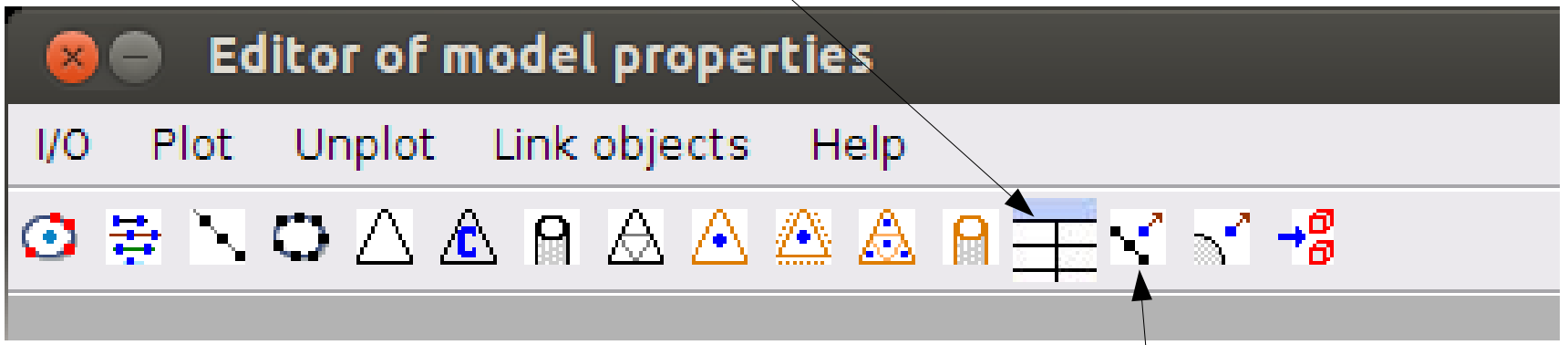
press [ok]

Dynamo recognizes the linked model. Now we can go back to the model editor for `myParticles`

In the editor, now we see that our model of isolated particles has a new field for the backbonemodel that is linked to it.

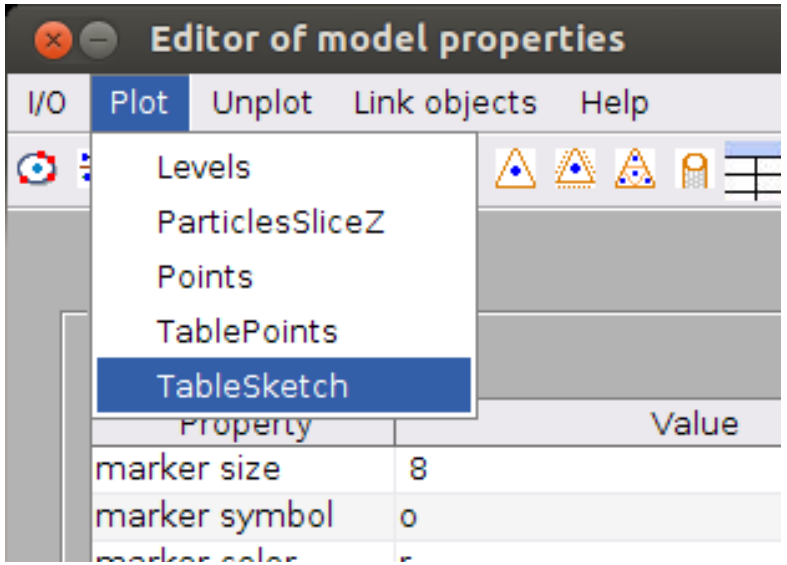


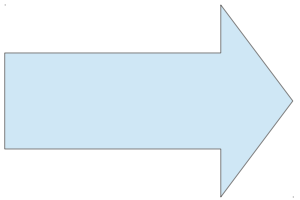
we renew now the table in our model....

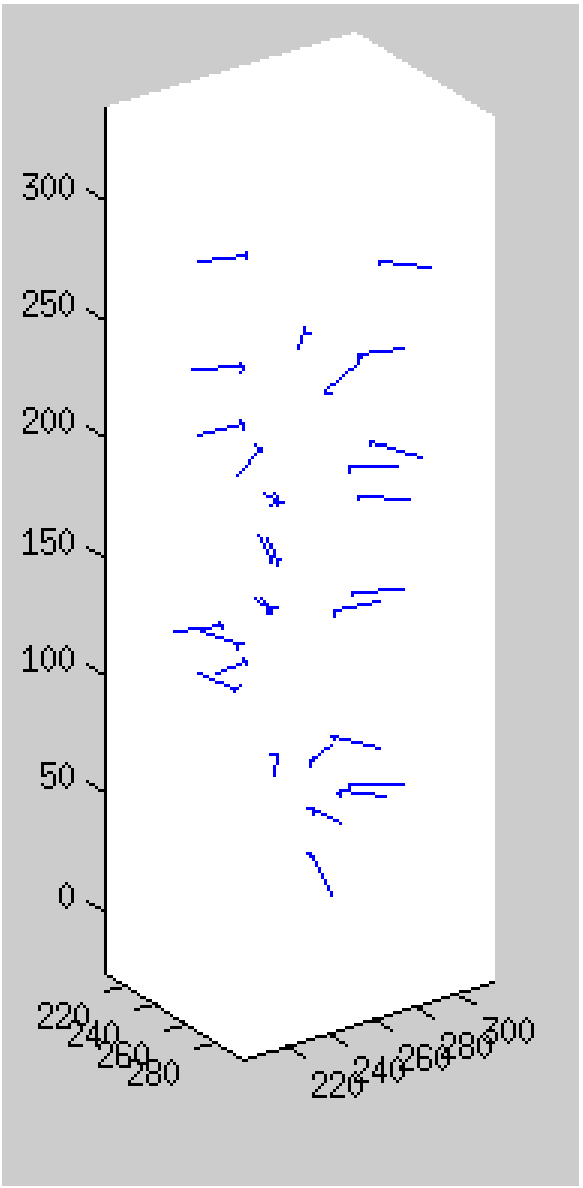


…. and set explicitely the particles with orientation normal to the backbone

before cropping the particles, we can check that the table looks ok.

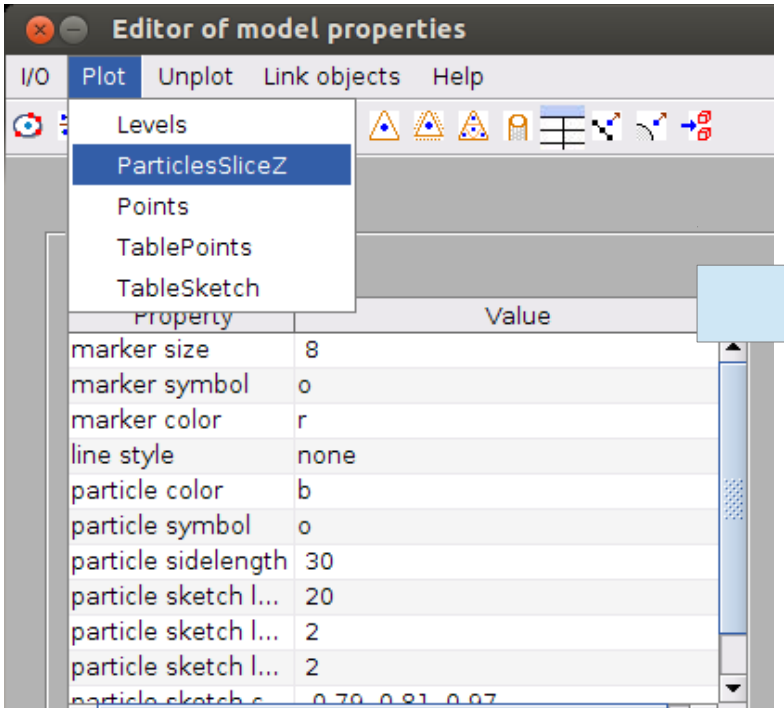


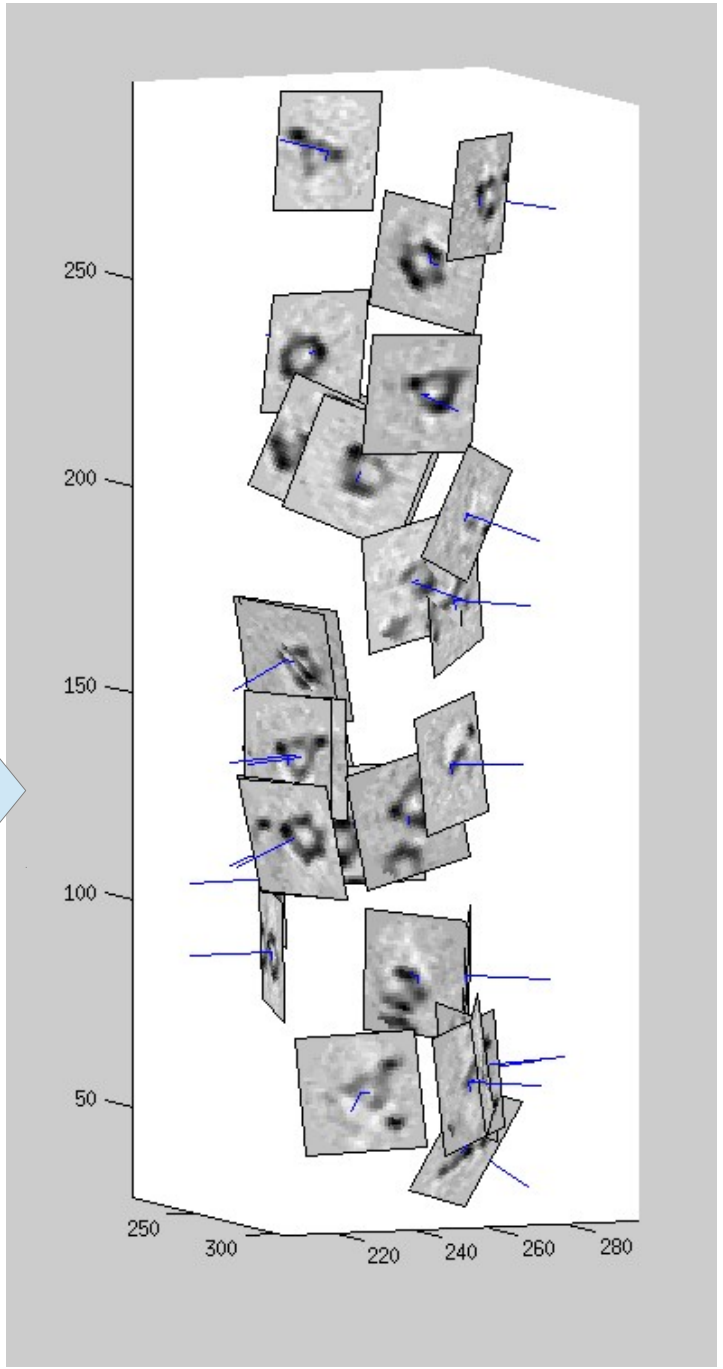




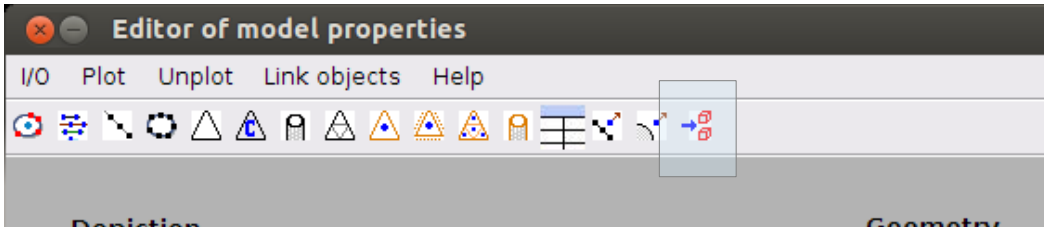
indeed the particles are oriented outwards from the central axis

other plotting option is the depiction of each particle as a projection on z with the right orientation.

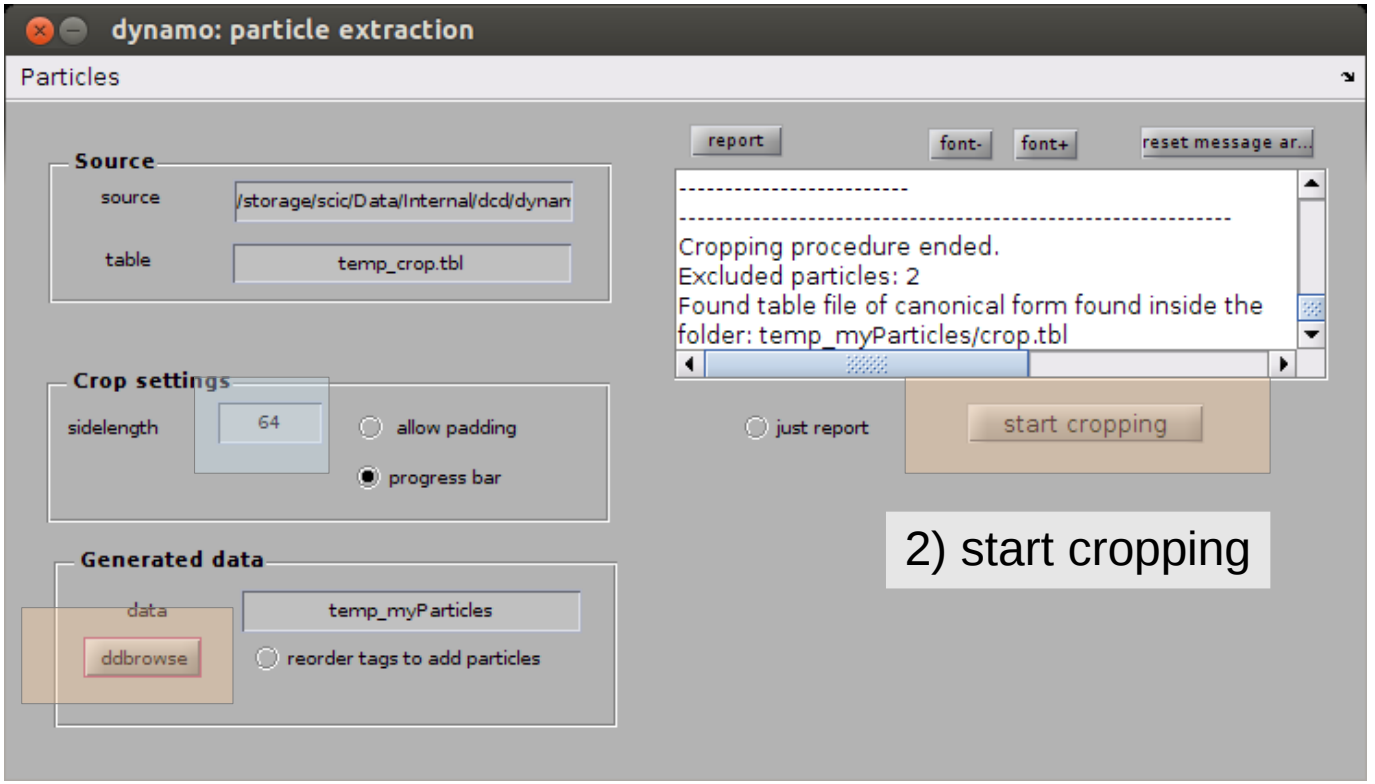




So, we can crop again our particles, but now with the right orientations

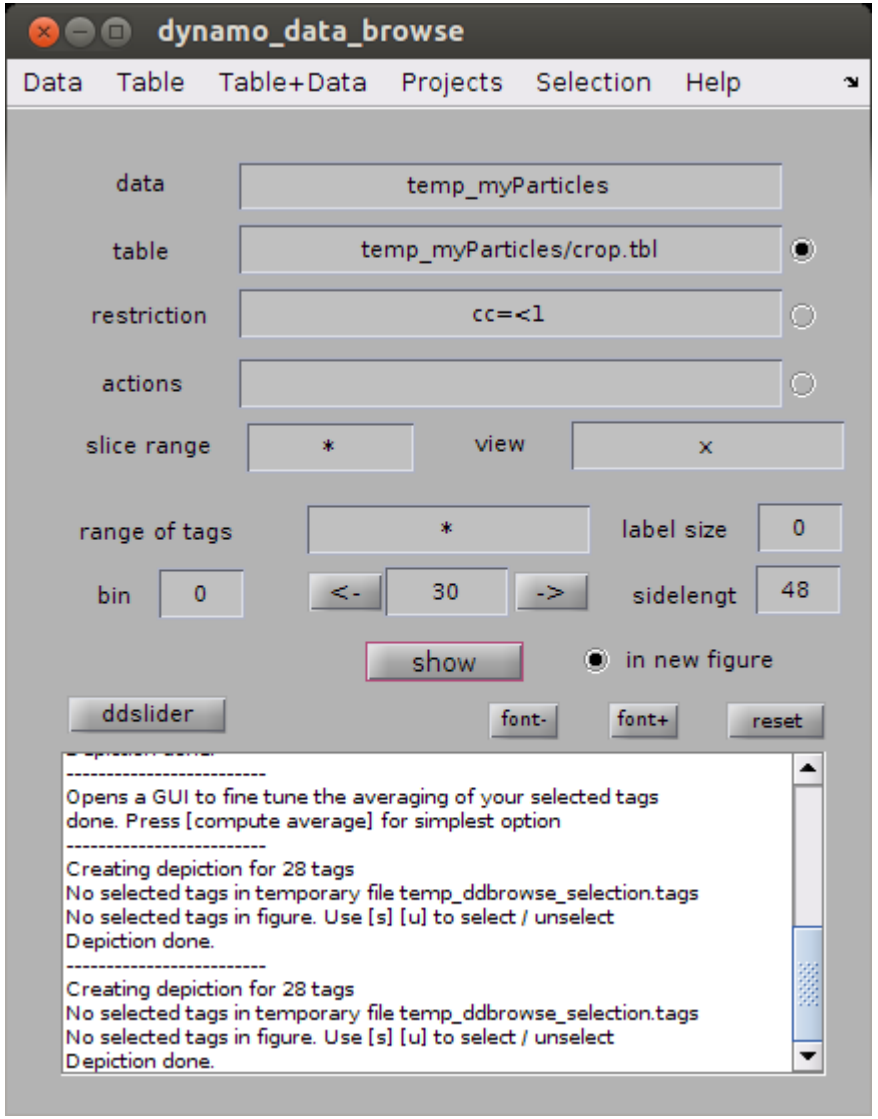


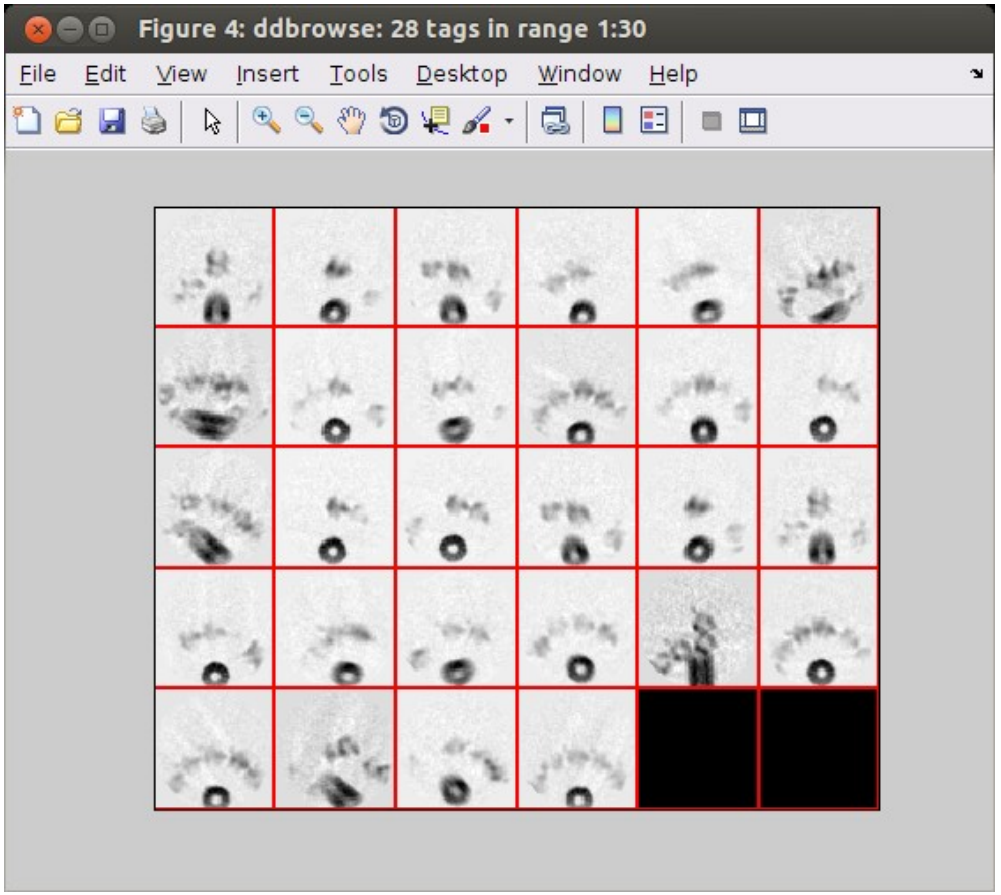
1) set size



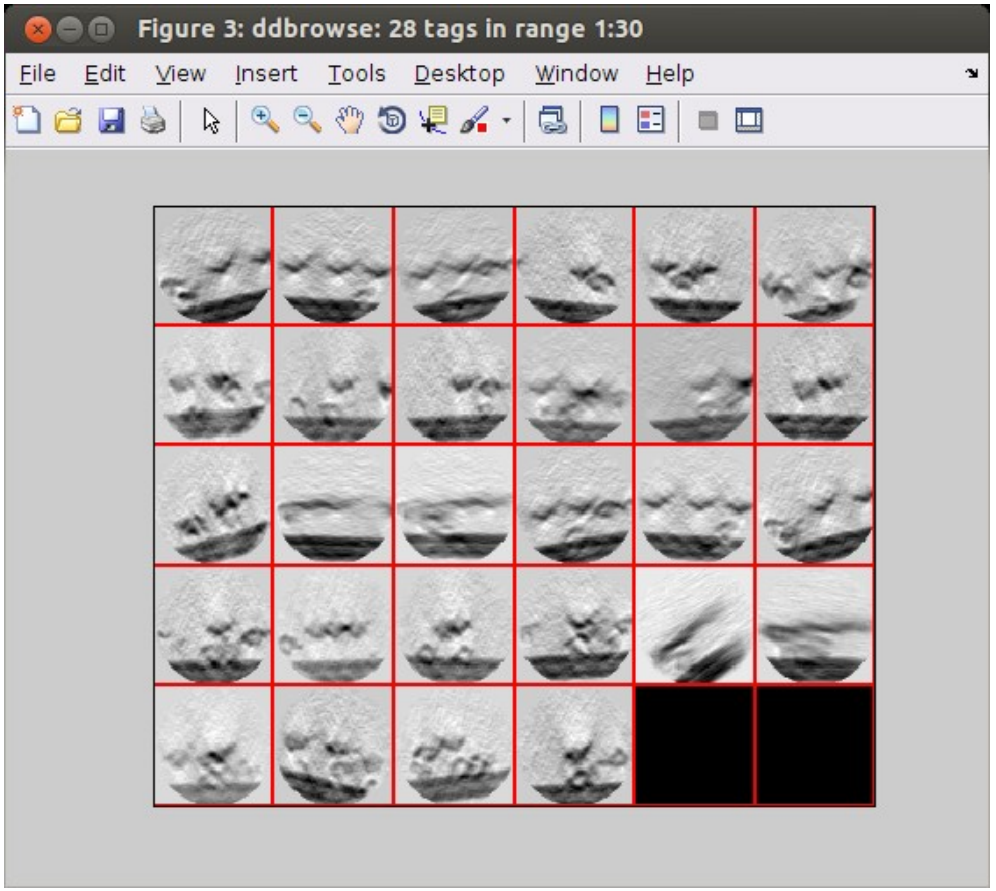
2) start cropping

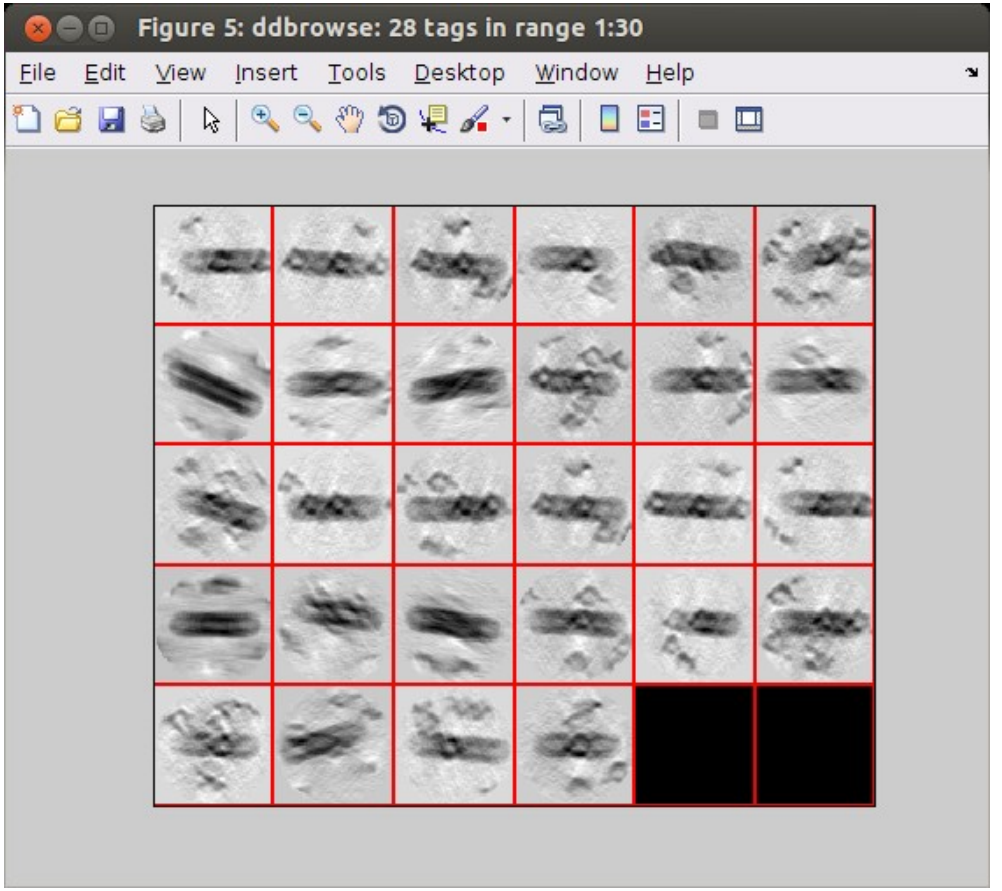
3) explore results





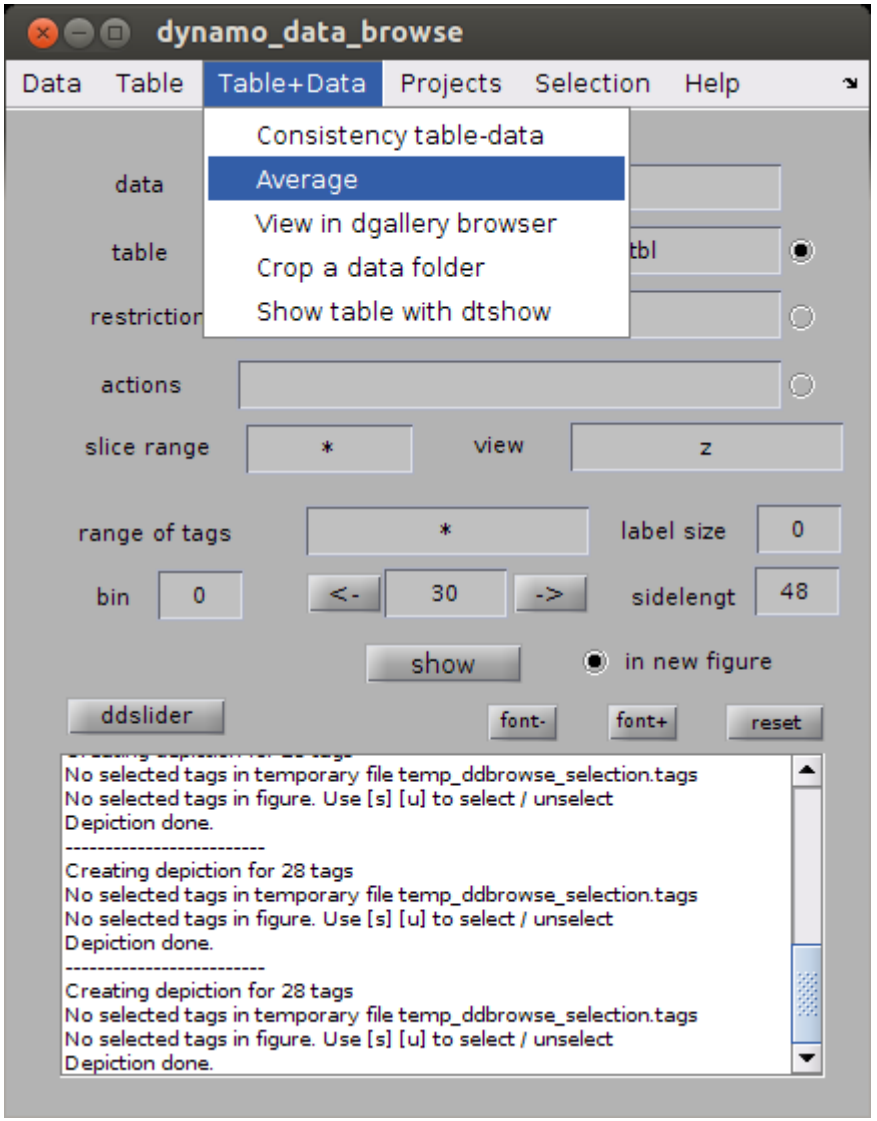
From the x projection view we see particles aligned in the direction of the filaments

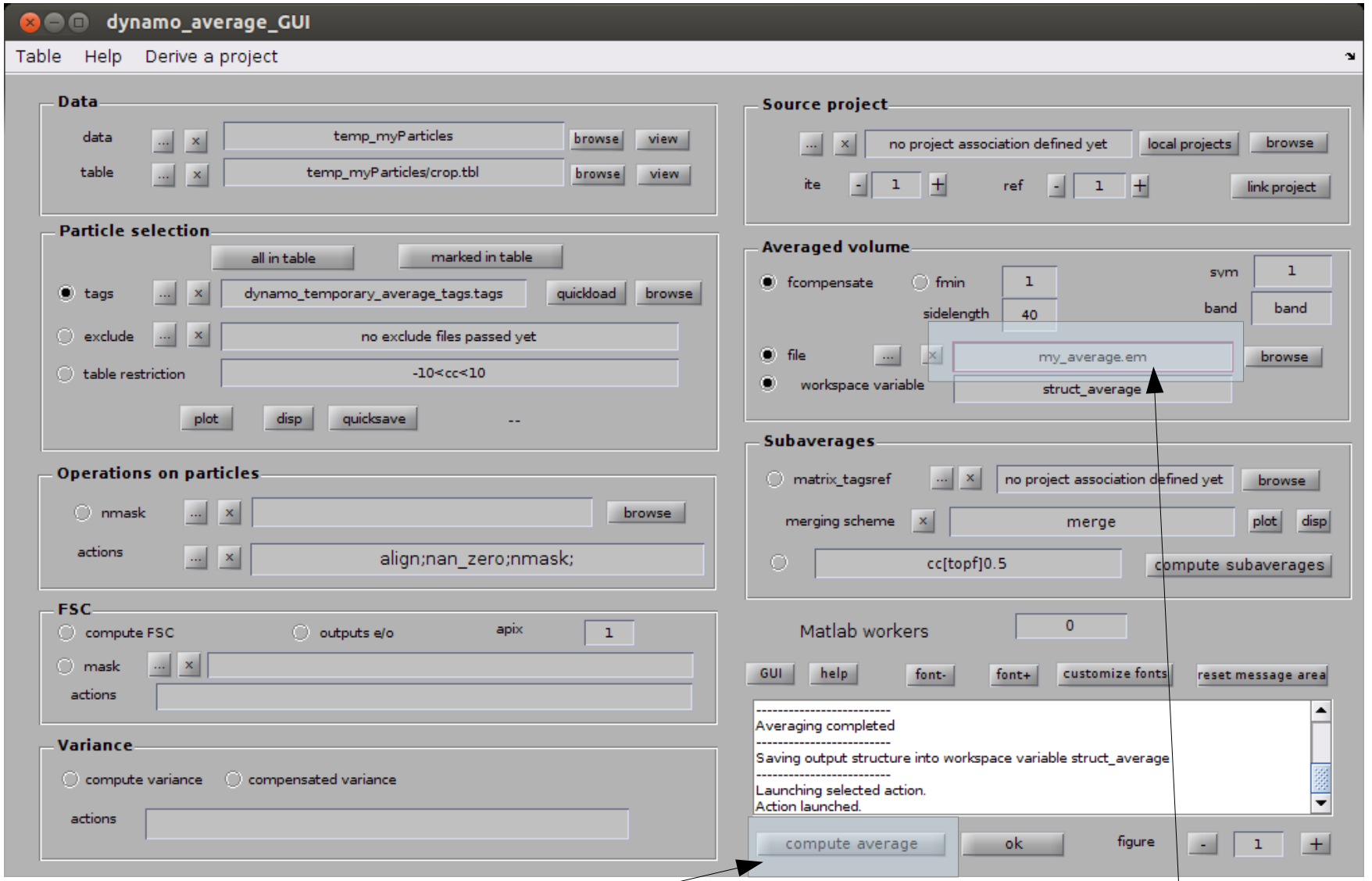




attention: the y and z views show that our manual picking hits the overall correct orientation, but obviously in a rather coarse way.

so, let's try to align them. First we create an average:

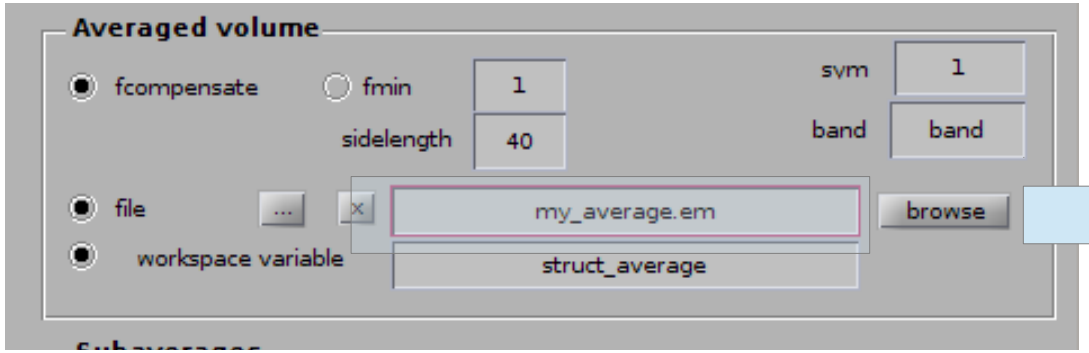




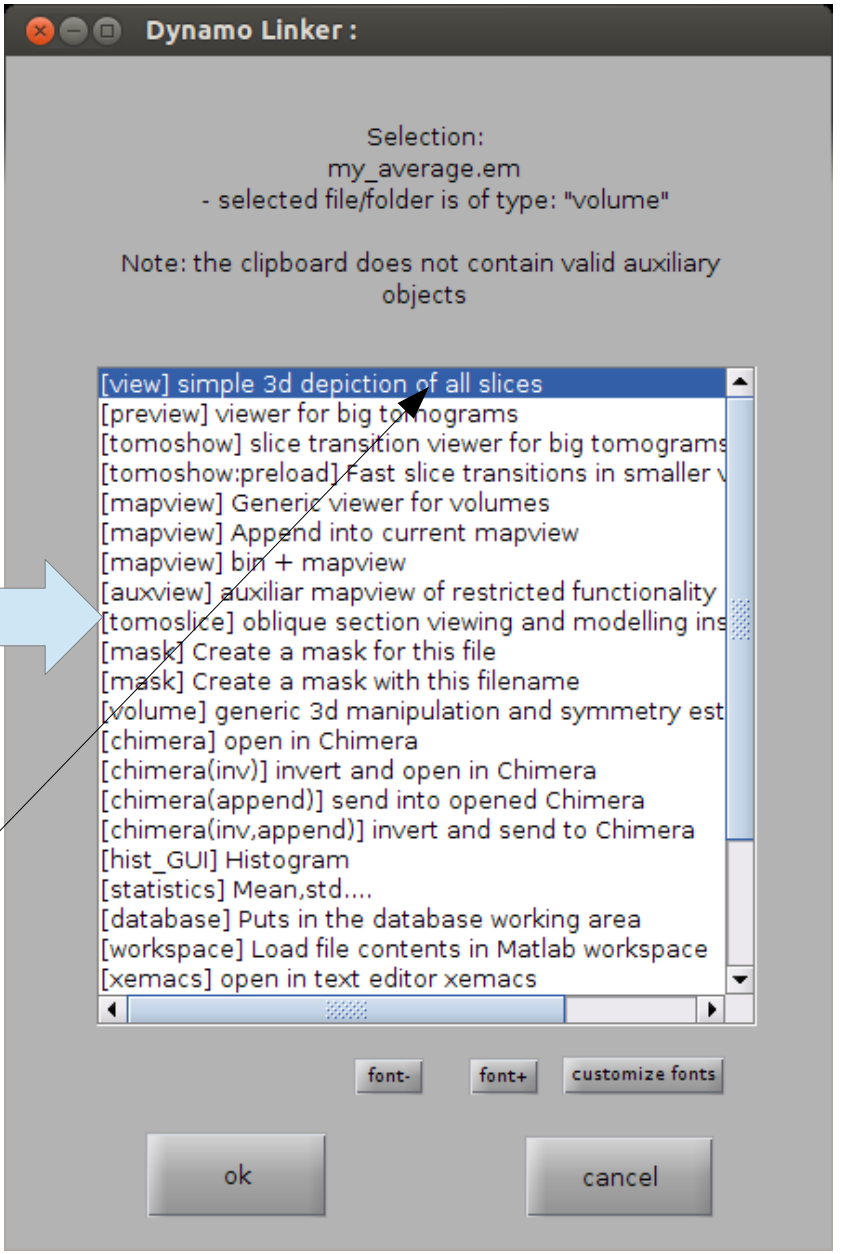
Press to start

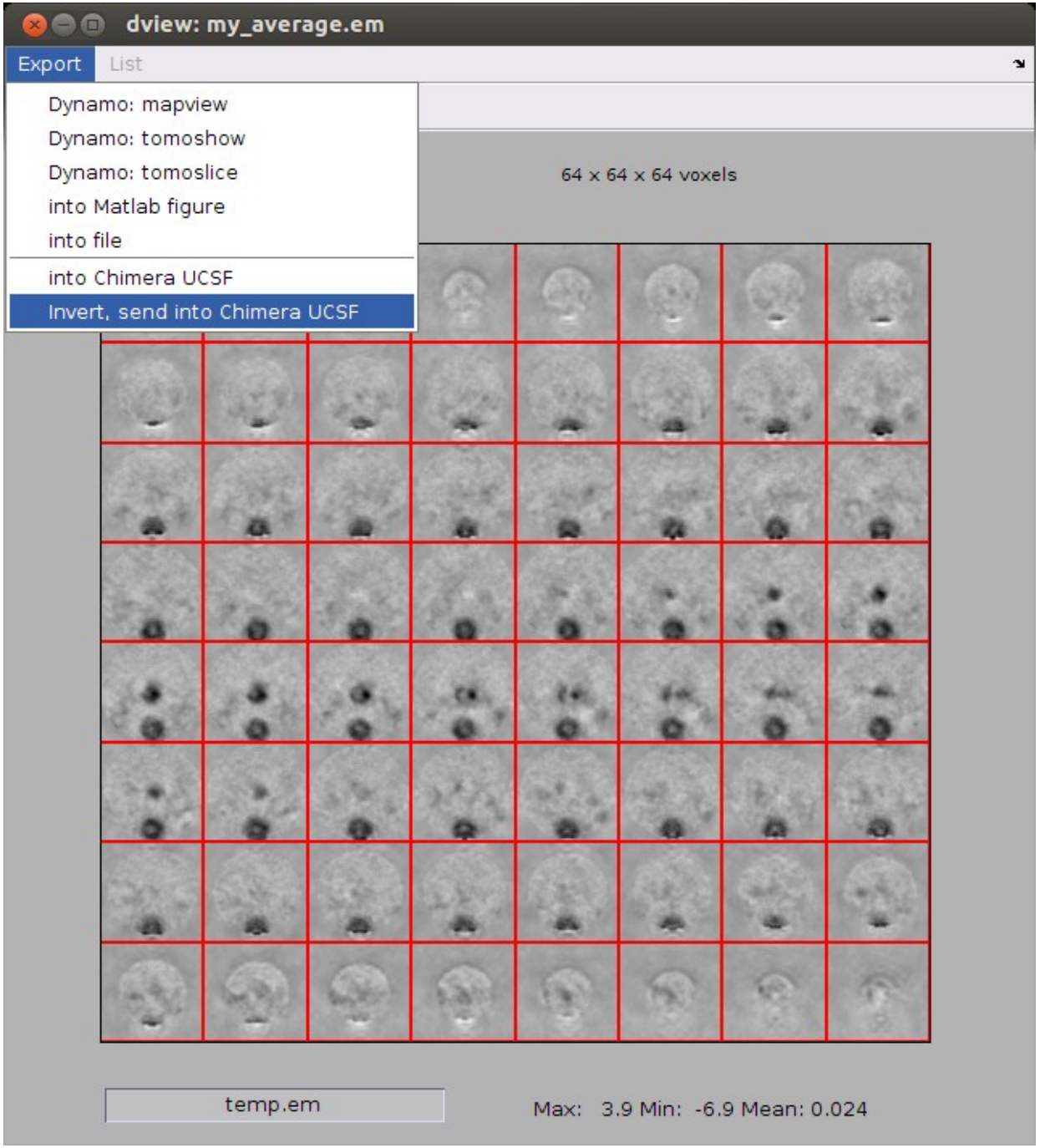
Result will be stored in this file

secondary click when the cursor is on the name of the file opens a menu of possible actions.

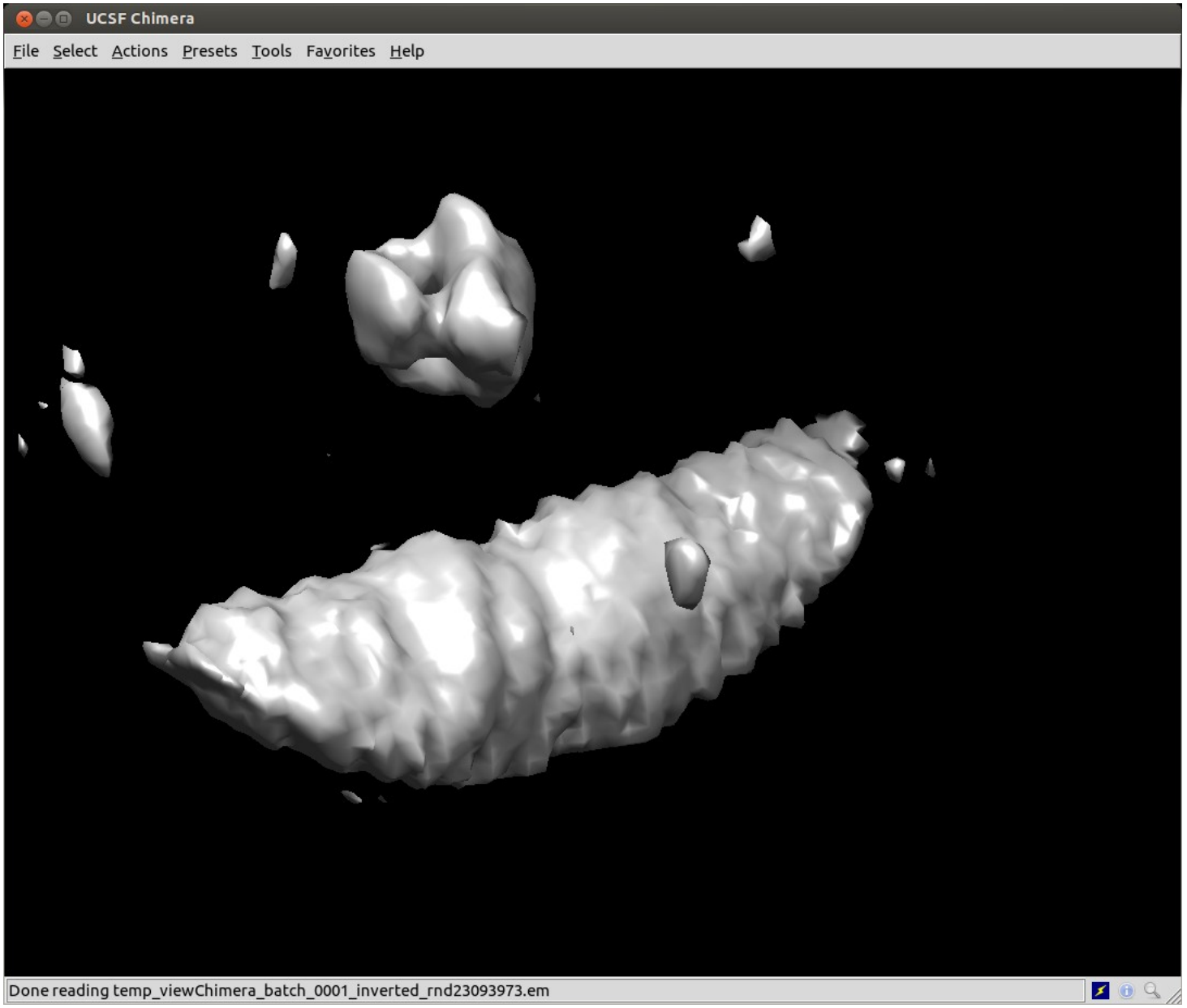


`dview` is a lightweight browser for subtomograms

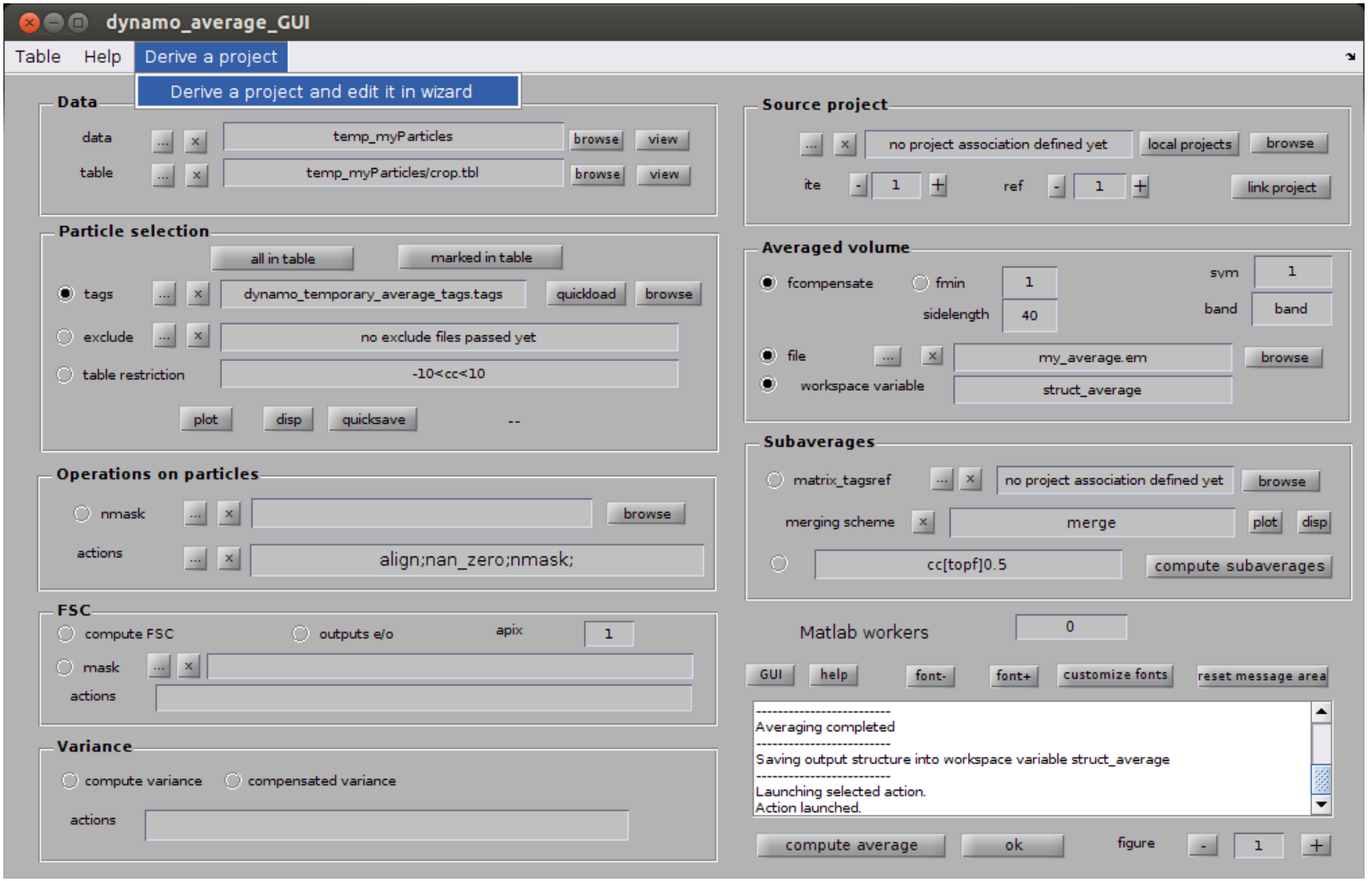




…. or send into Chimera from this browser


UCSF Chimera
File Select Actions Presets Tools Favorites Help
Done reading temp_viewChimera_batch_0001_inverted_rnd23093973.em

We can create a project prototype for this combination of table, data and initial average

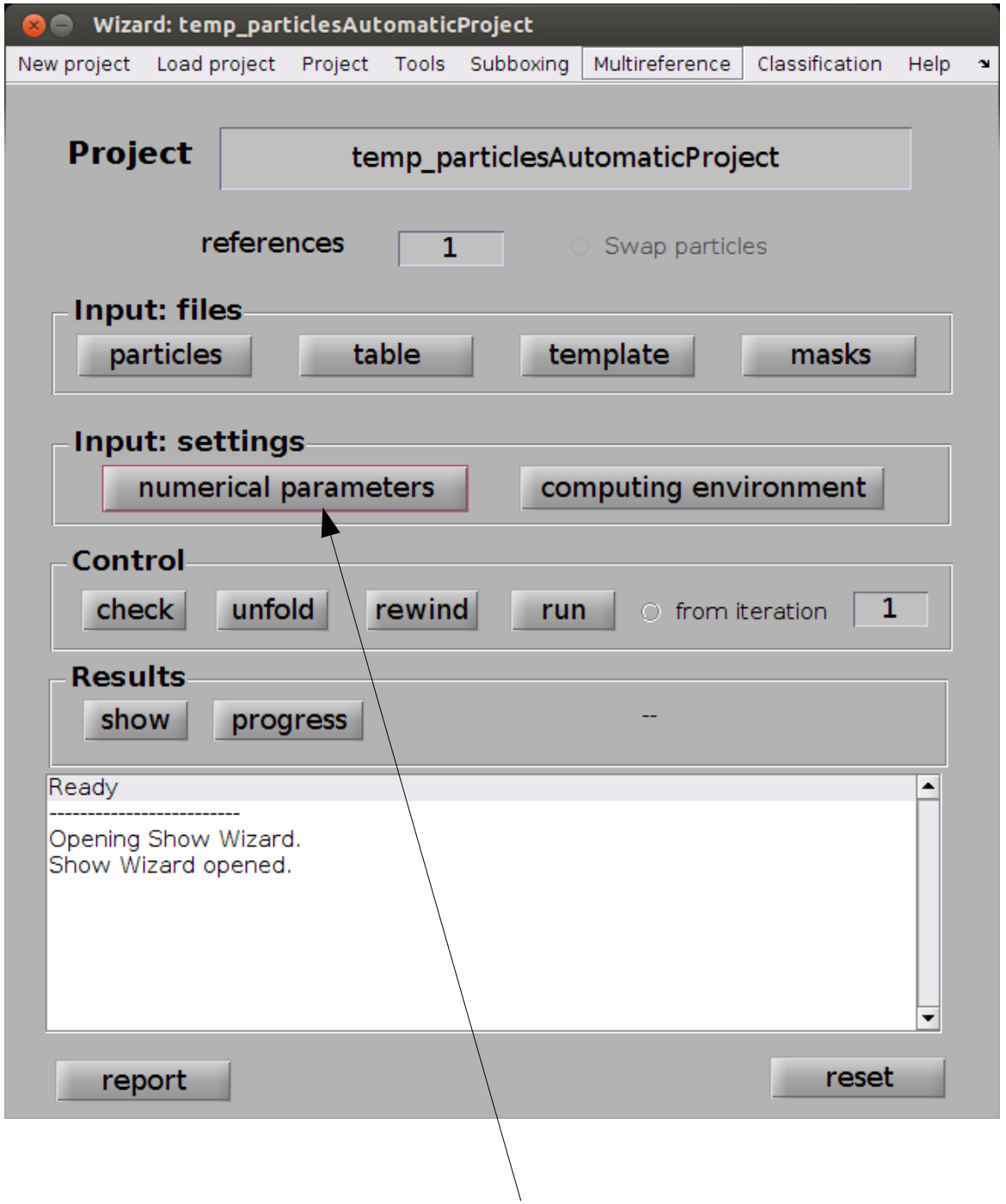


The project has been created in disk.

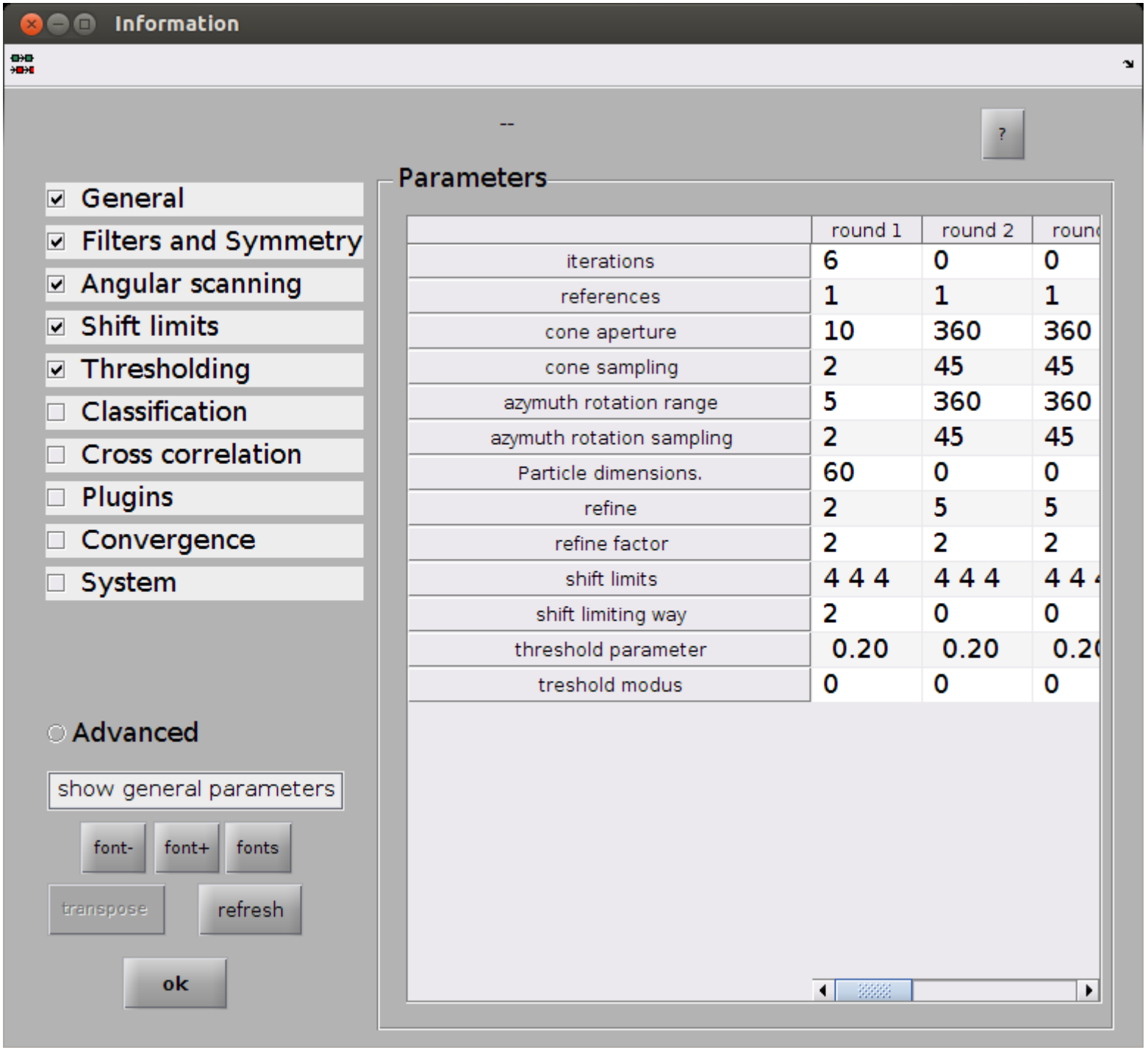
The settings of this default project are:

- Table and data set are inherited form the average GUI
- Initial reference is the computed average
- Mask and classification mask are simple spheres.
- Fourier mask is assumed full
- Angular scannig parameters are tuned to produce a rather local search.

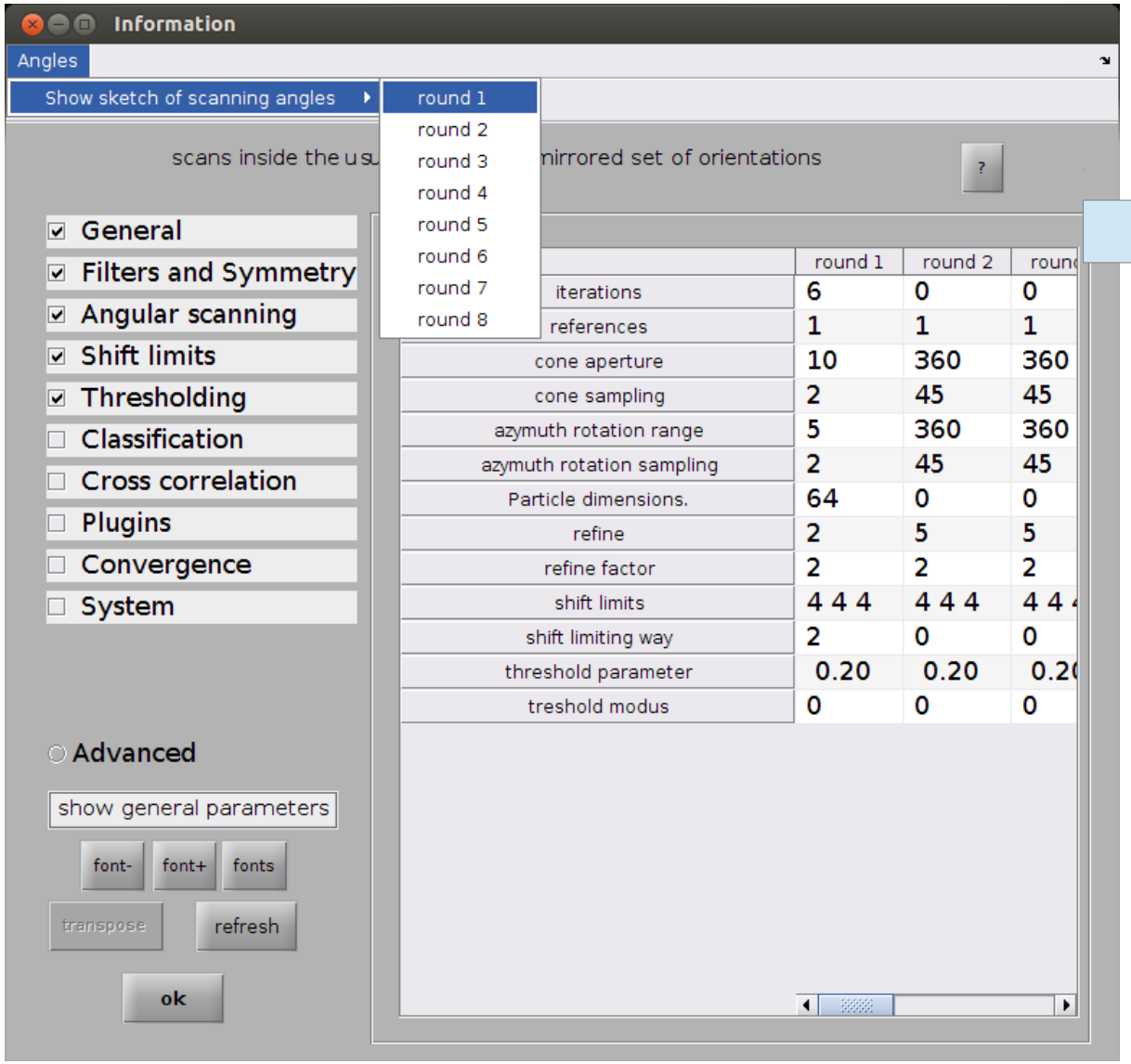
The Wizard GUI pops up for further operations.
.
You could use it to tune the project for specific needs,
although we will just use the default options.

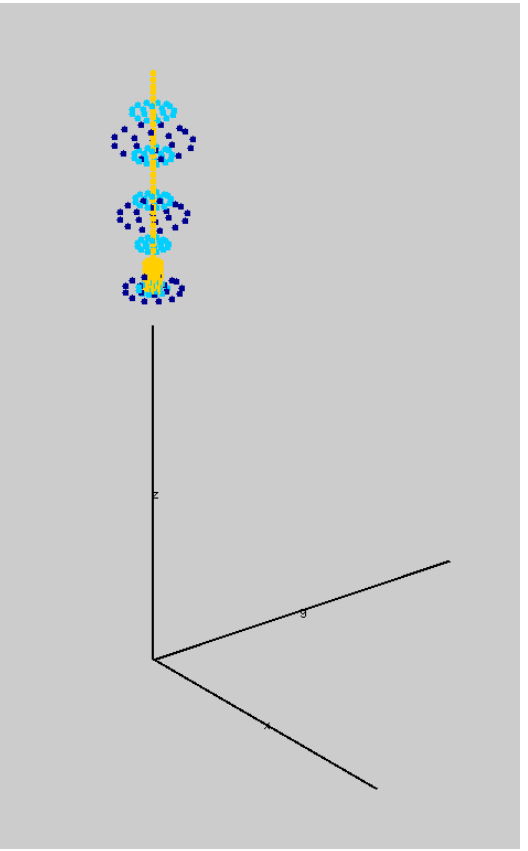


You can check the numerical parameters that were generated automatically

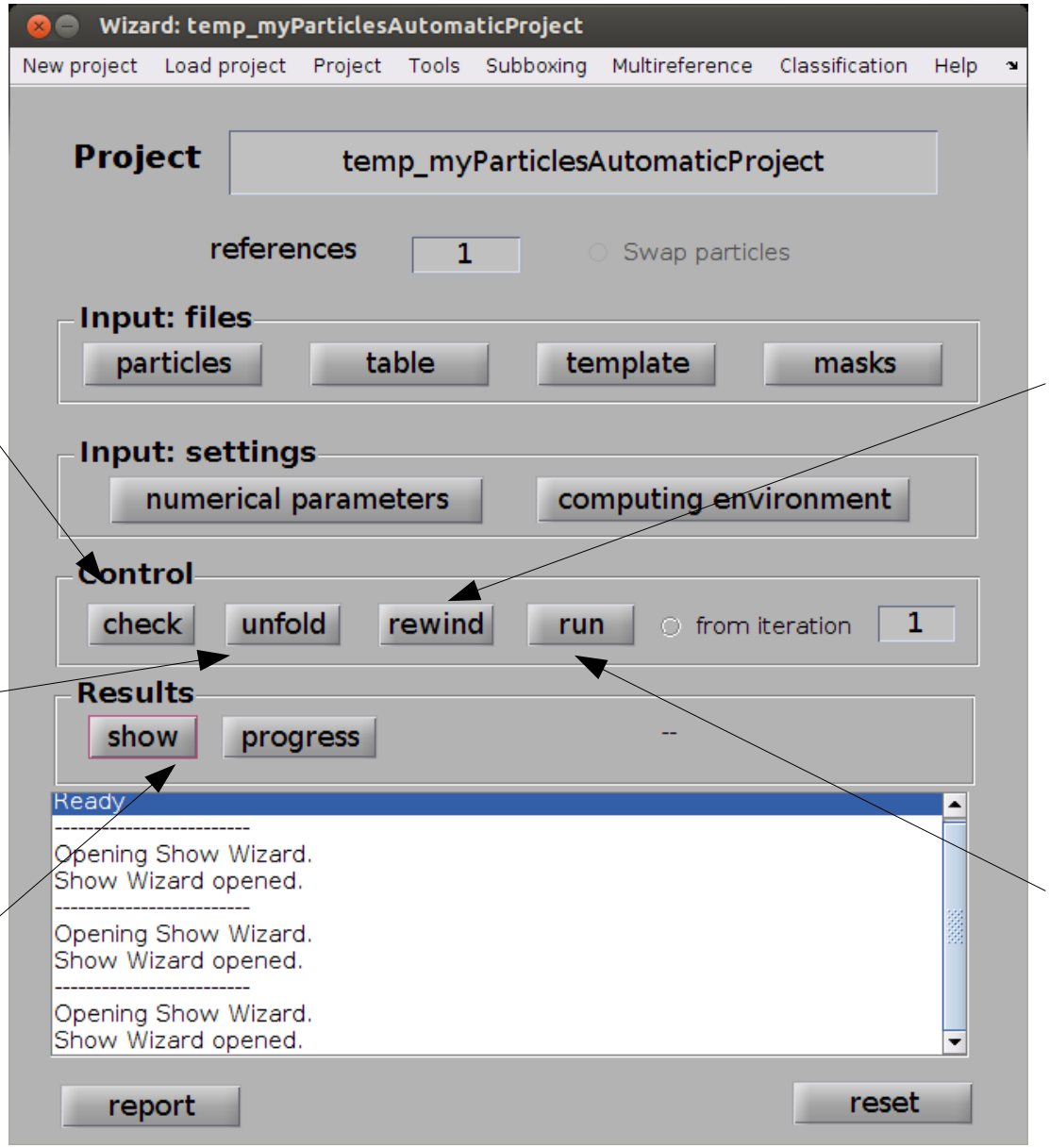

Information
--
?
General
Filters and Symmetry
Angular scanning
Shift limits
Thresholding
Classification
Cross correlation
Plugins
Convergence
System
Advanced
show general parameters
font-
font+
fonts
transpose
refresh
ok
Parameters
round 1
round 2
iterations 6 0 0
references 1 1 1
cone aperture 10 360 360
cone sampling 2 45 45
azymuth rotation range 5 360 360
azymuth rotation sampling 2 45 45
Particle dimensions. 60 0 0
refine 2 5 5
refine factor 2 2 2
shift limits 4 4 4 4 4 4
shift limiting way 2 0 0
threshold parameter 0.20 0.20
treshold modus 0 0 0

You can check the scanning geometry induced by these parameters.





a very local search around the axial directions coded in the initial table



Check
looks for inconsistencies in the parameters

Unfold
prepares the project for execution

Rewind
prepares a project to be executed again

Run
executes the project.

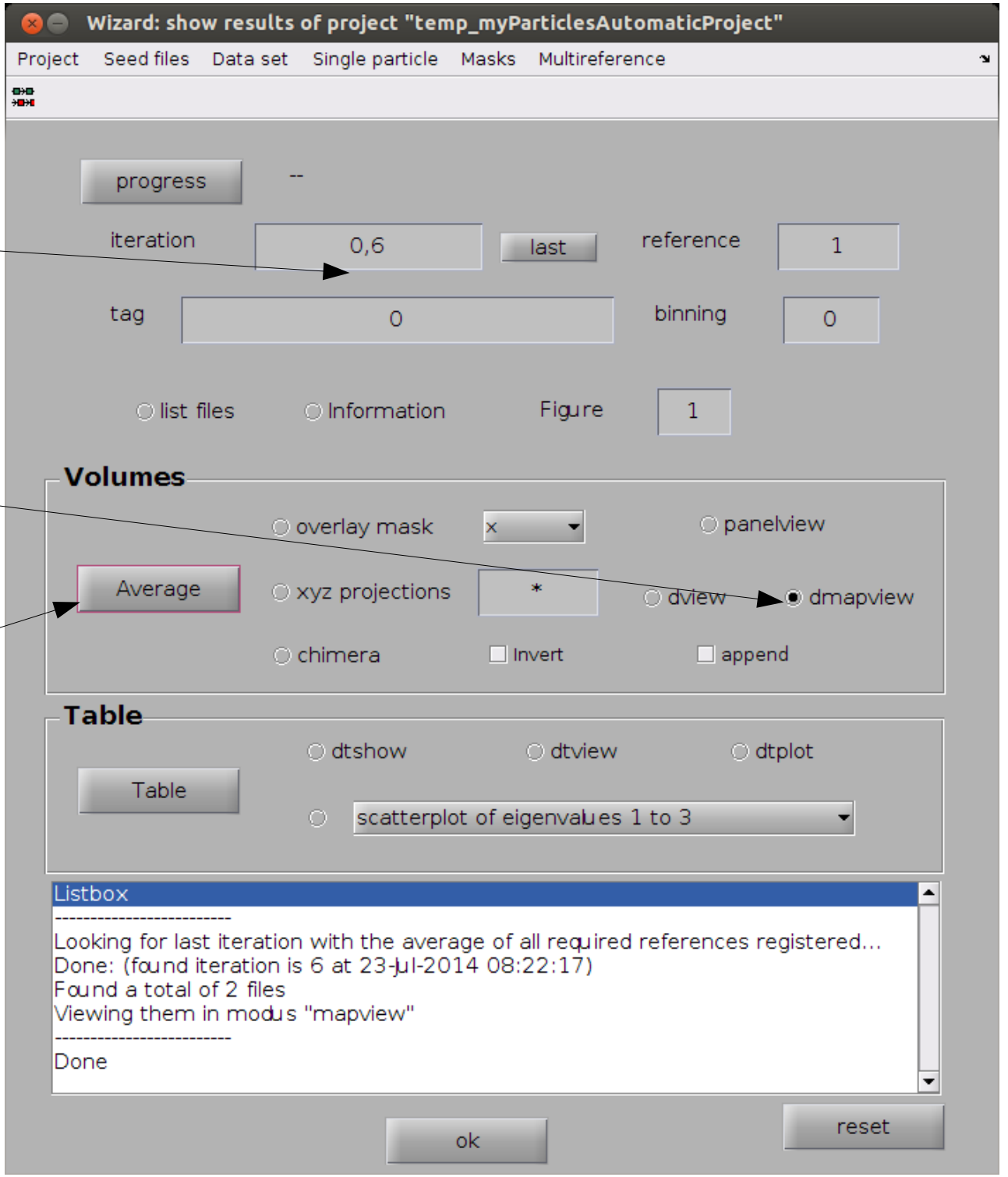
Show
Opens the Wizard show: a panel that allows accessing the results of the alignment in diffferent graphical formats

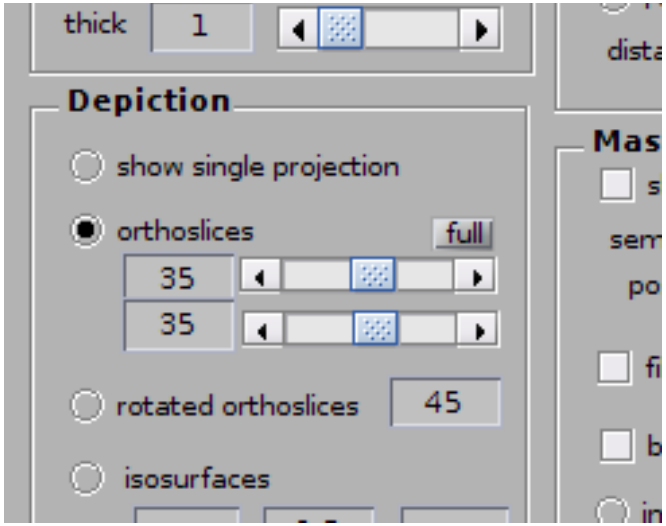
in the Wizard: show Panel

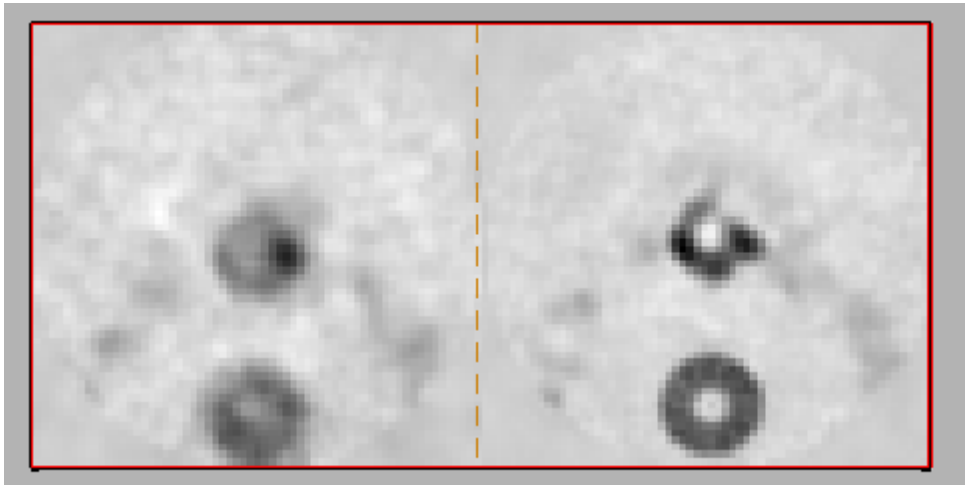
compare the last iteration
with the provided template

choose mapview for visualization

show the plot







iteration 0: provided template

iteration 6:

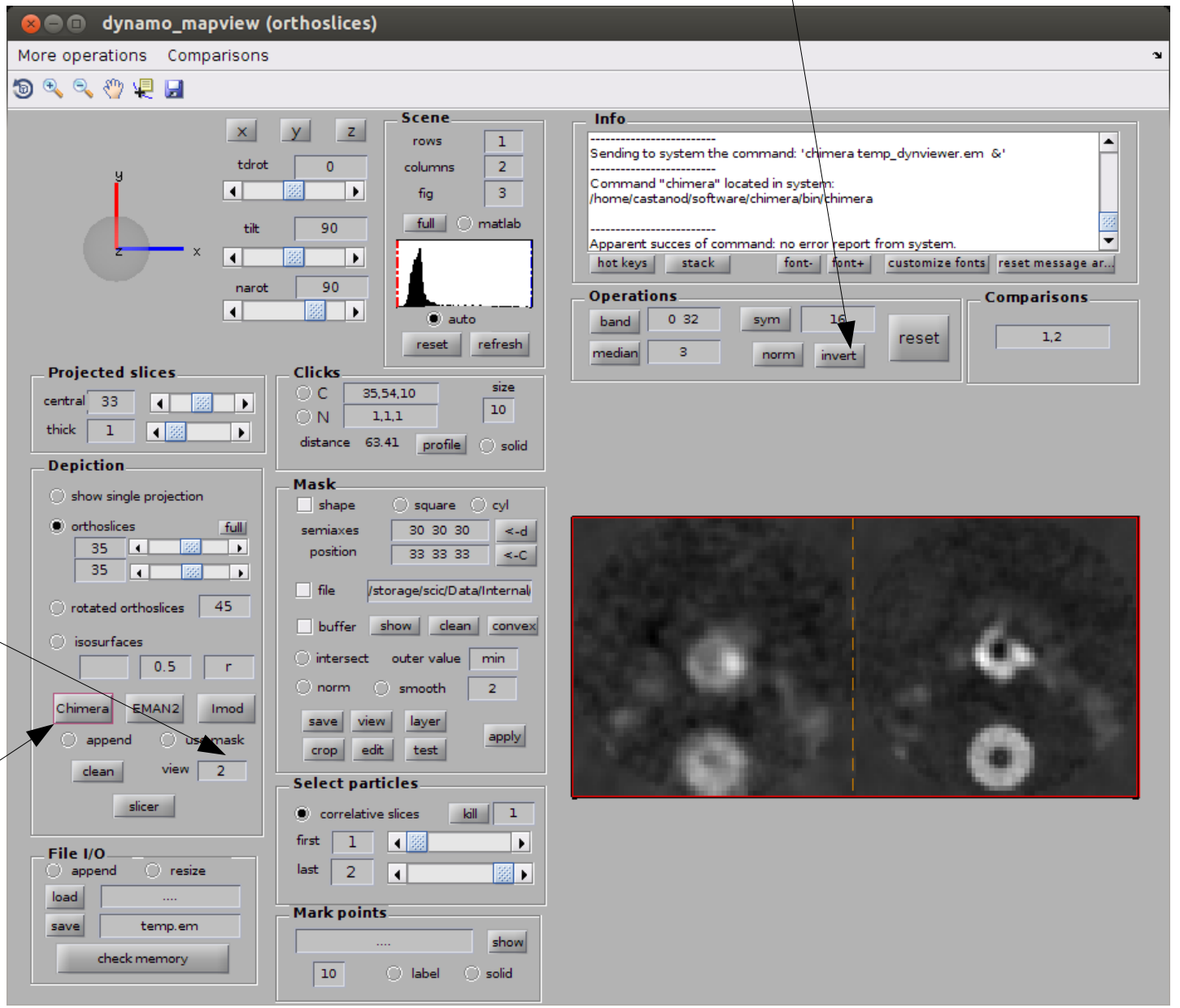
you can focus on different slices with the controls on the depiction panel
or the hotkeys ([c] for instance chooses the slice under the curso)

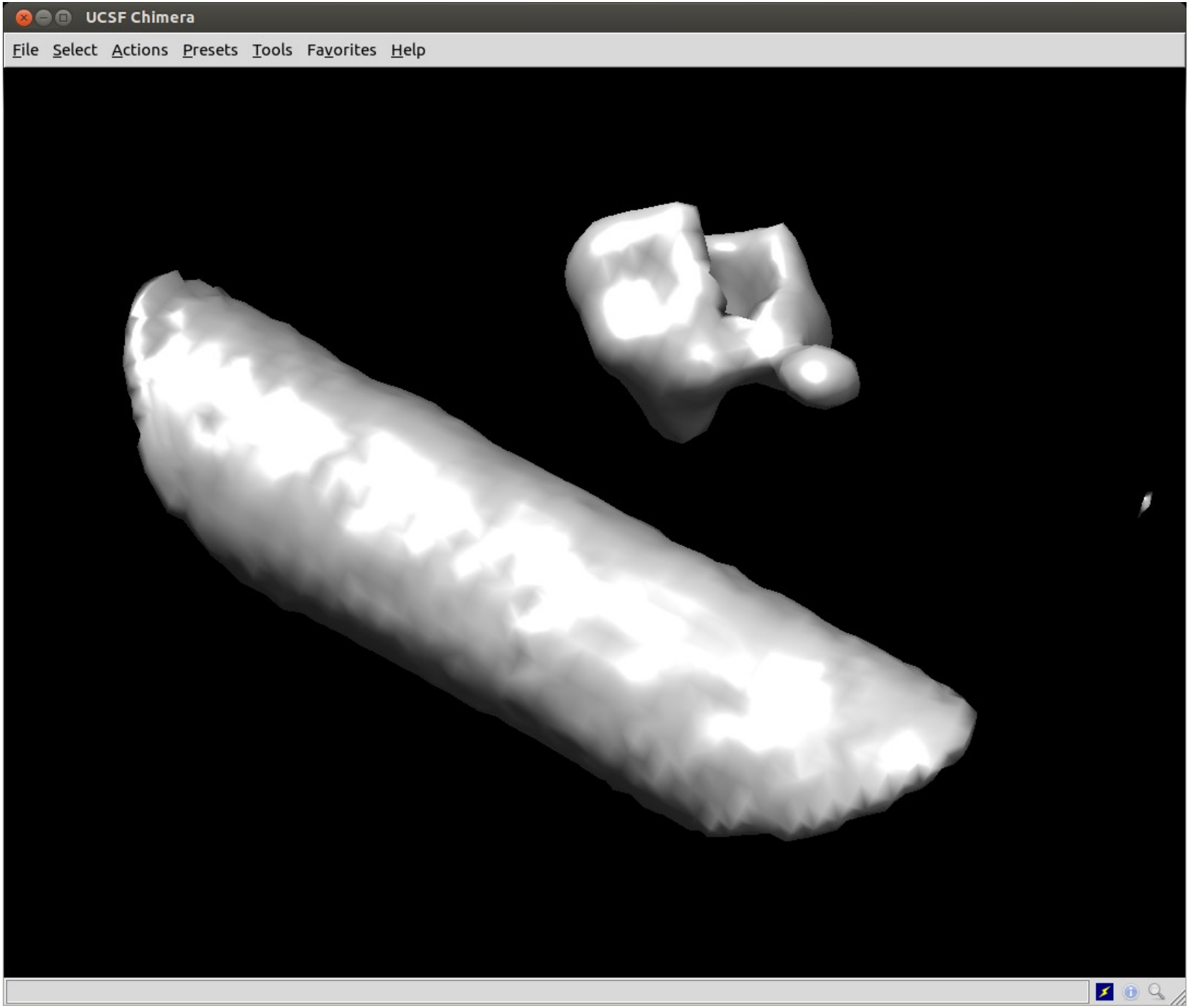
To see the result of the iteration in Chimera

invert the contrast

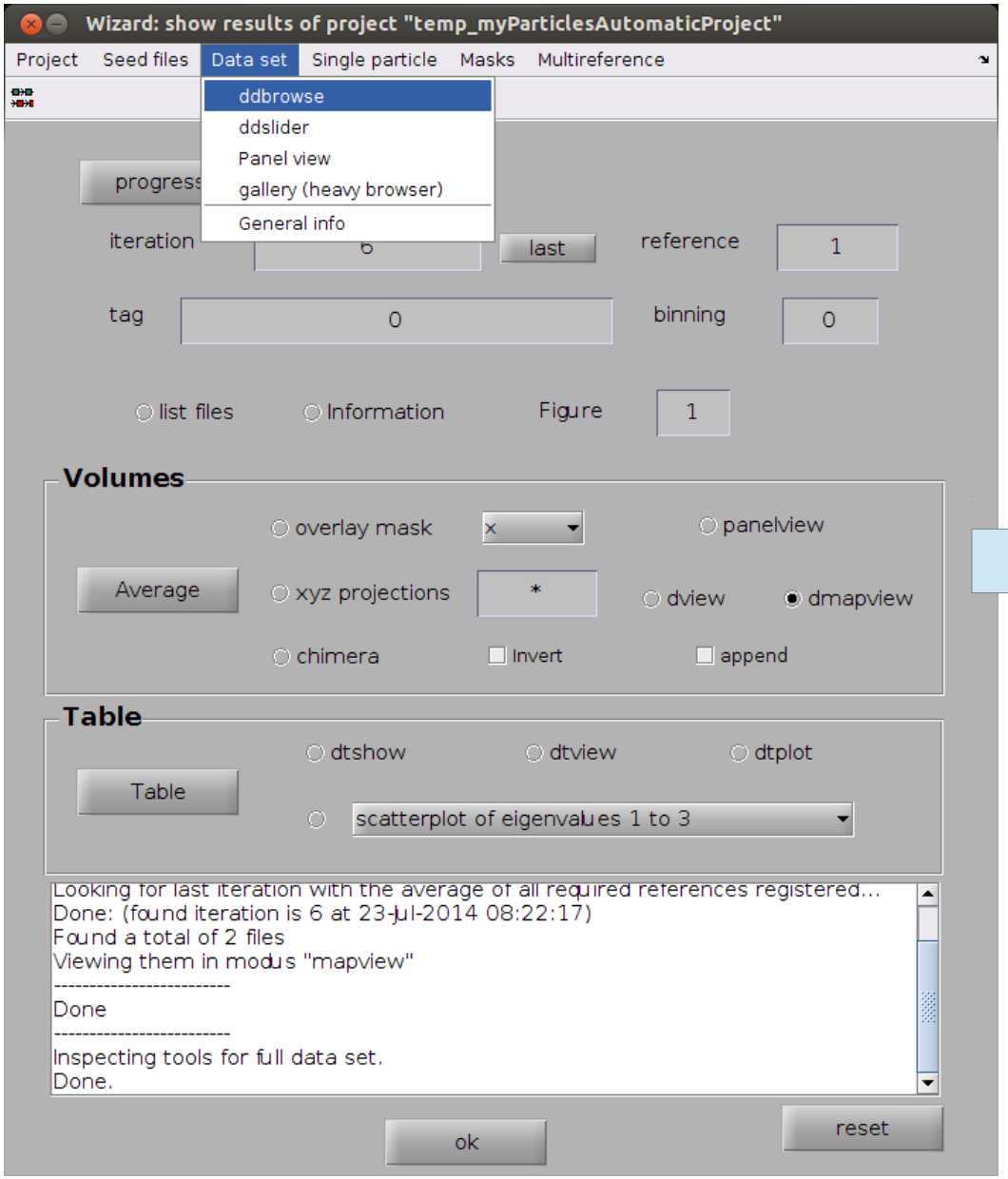
choose the second volume

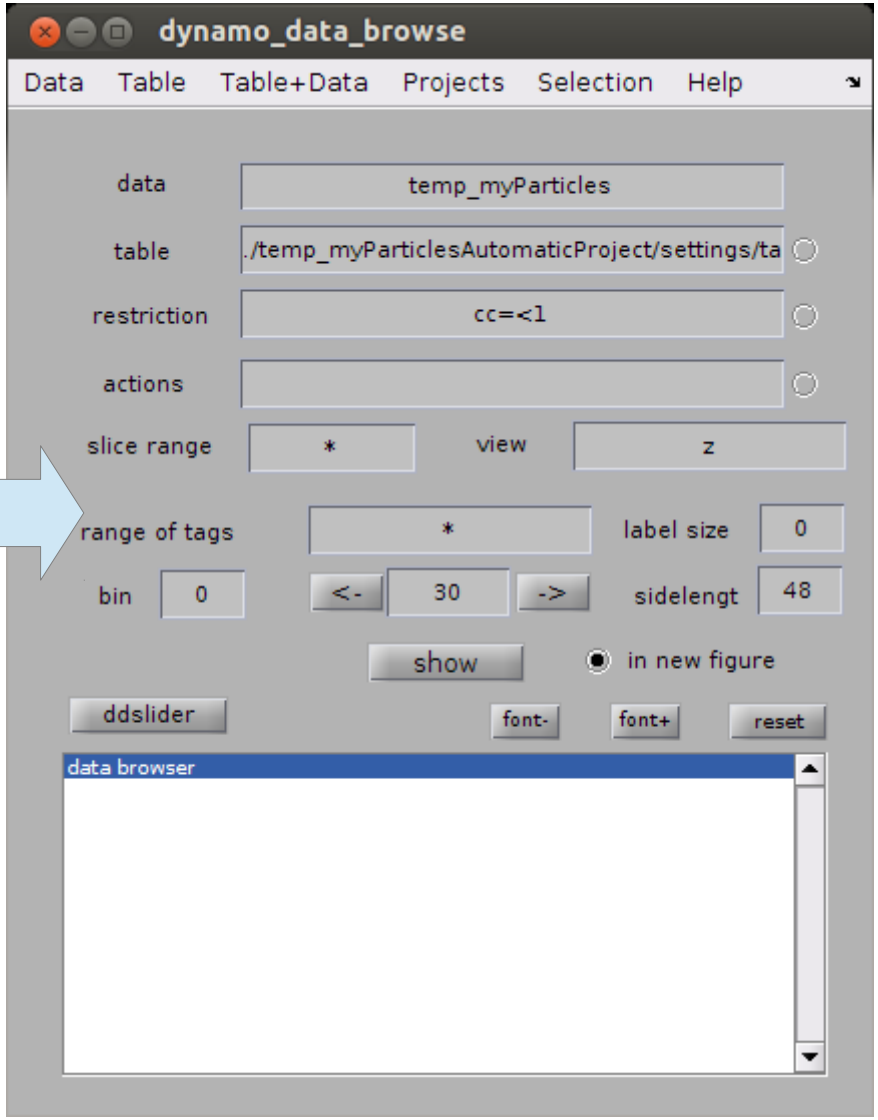
and press [Chimera]

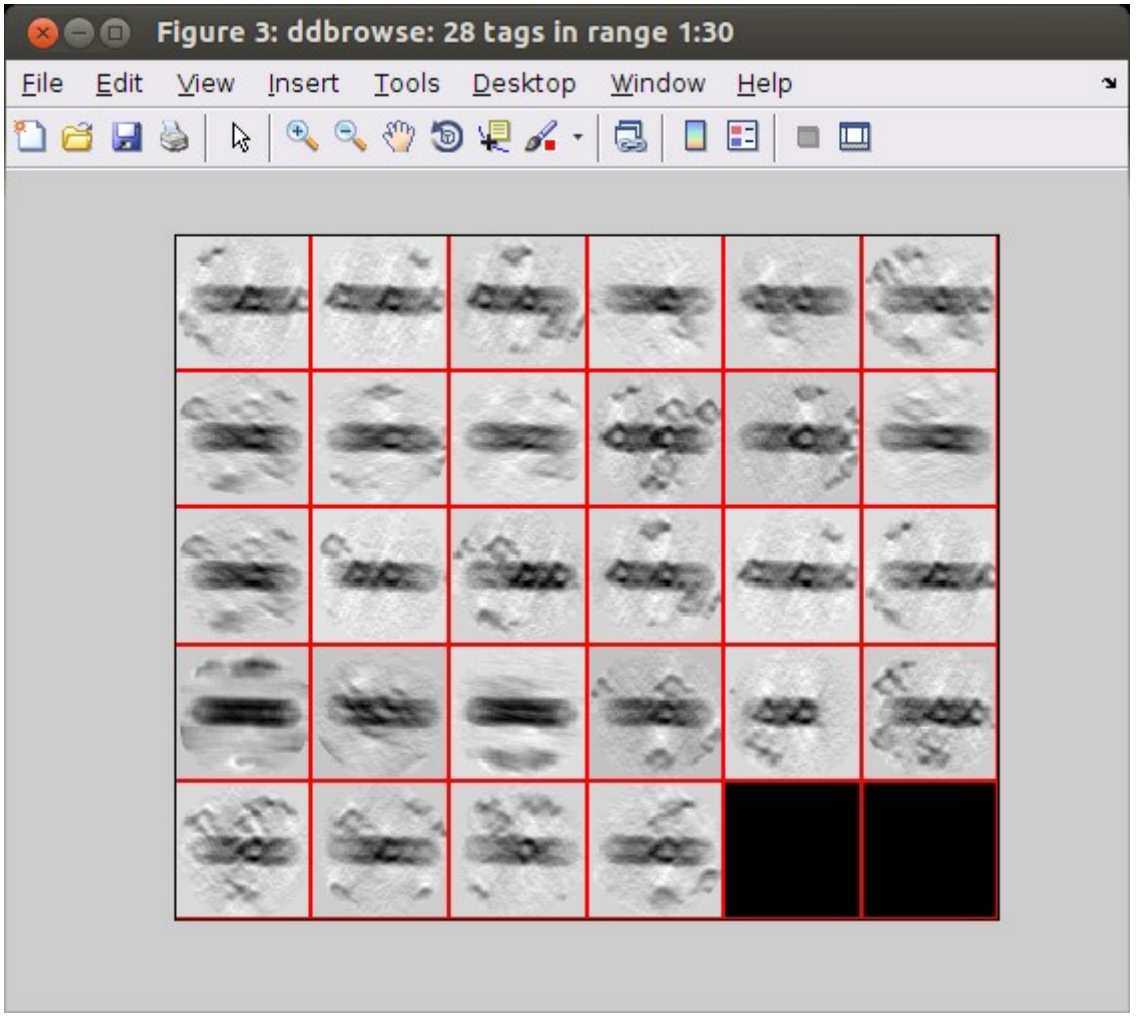



UCSF Chimera
File Select Actions Presets Tools Favorites Help

we can also check the a parameters on the particles themselves







particles are now coherently aligned