Coot Tutorial

CCP4 Workshop

April 8, 2005

Contents

1 Mousing

First, how do we move around and select things?

Left-mouse Drag	Rotate view
Ctrl Left-Mouse Drag	Translates view
Shift Left-Mouse	Label Atom
Right-Mouse Drag	Zoom in and out
Middle-mouse	Centre on atom
Scroll-wheel Forward	Increase map contour level
Scroll-wheel Backward	Decrease map contour level

2 First Steps

In this tutorial, we will learn how to do the following:

1. Start Coot
2. Display coordinates
3. Display a map
4. Zoom in and out
5. Recentre on Different Atoms
6. Change the Clipping (Slab)
7. Recontour the Map
8. Change the Map Colour
9. Display rotamers and refine residue

2.1 Get the files

Before we start, let's get the files on which we will be working:

```
$ wget http://www.ysbl.york.ac.uk/~emsley/coot/tutorial/tutorial.pdb
$ wget http://www.ysbl.york.ac.uk/~emsley/coot/tutorial/rnasa-1.8-all_refmac1.mtz
```

In York:
`/y/people/emsley/tutorial/tutorial.pdb`
and
`/y/people/emsley/tutorial/rnasa-1.8-all_refmac1.mtz`

2.2 Start Coot

Before you start `coot`, you need to setup the proper environment. This is currently done by "sourcing" a setup file. The location of this setup file is system dependent, here (in York) you need to type:

```
$ source /y/people/emsley/setup/ccp4
$ source /y/people/emsley/setup/coot
```

To then use coot, simply:
`$ coot`
When you first start `coot`, it should look something like Figure 1.

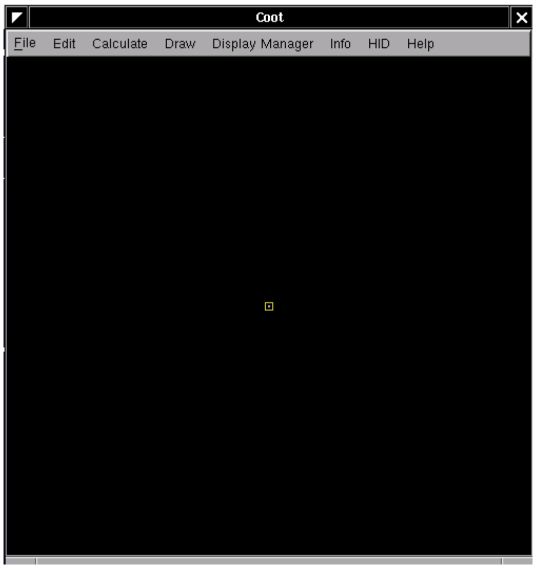

Figure 1: Coot at Startup
Not much to see at present... Actually, from now coot screenshots will be displayed with a white background, whereas you will see a black one

2.3 Display Coordinates

So let's read in those coordinates:

- Select "File" from the Coot menu-bar[1]
- Select the "Open Coordinates" menu item
  [Coot displays a Coordinates File Selection window]
- Either
  - Select `tutorial.pdb` from the "Files" list

  or

  - Type `demo.pdb` in the Selection: entry
- Click "OK" in the Coordinates File Selection window
  [Coot displays the coordinates in the Graphics Window]

2.4 Adjust Virtual Trackball

By default, Coot has a "virtual trackball" to relate the motion of the molecule to the motion of the mouse. Many people don't like this.

So you might like to try the following:
In the Coot main menu-bar:
HID → Virtual Trackball → Flat
(Use the "Spherical Surface" option to turn it back to how it is by default)

[1]Note you can also use "Alt-F" instead of clicking on "File"

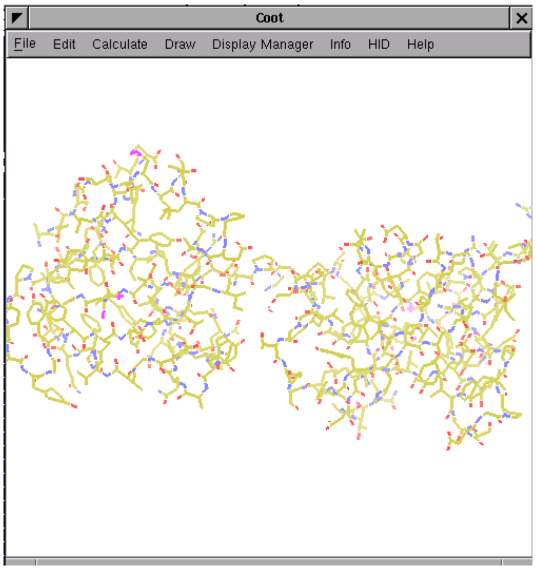

Figure 2: Coot After Loading Coordinates

2.5 Display maps

We are at the stage where we are looking at the results of the refinement. The refinement programs stores its data (labelled lists of structure factor amplitudes and phases) in an "MTZ" file. Let's take a look...

- Select "File" from the Coot menu-bar
- Select "Auto Open MTZ" menu item *[Coot displays a Dataset File Selection window]*
- Select the filename `rnasa-1.8-all_refmac1.mtz`

If you choose instead "Open MTZ, cif or phs..." you will see:

- *[Coot displays a Dataset File Selection window]*
- Select the filename `rnasa-1.8-all_refmac1.mtz`

  [Coot displays a Dataset Column Label Selection window]

  Notice that you have a selection of different column labels for the "Amplitudes" and "Phases", however, let's use the defaults: "FWT" and "PHWT".
- Press "OK" in the Column Label Window

  Now open the MTZ file and select column labels "DELFWT" and "PHDELWT".

  So now we have 2 maps (whether auto-opened or not).

2.6 Zoom in and out

To zoom in, click Right-mouse and drag it left-to-right[2]. To zoom out again, move the mouse the opposite way.

[2]or up-to-down, if you prefer that

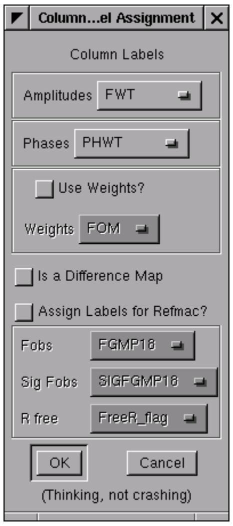

Figure 3: Coot MTZ Column Label Selection Window

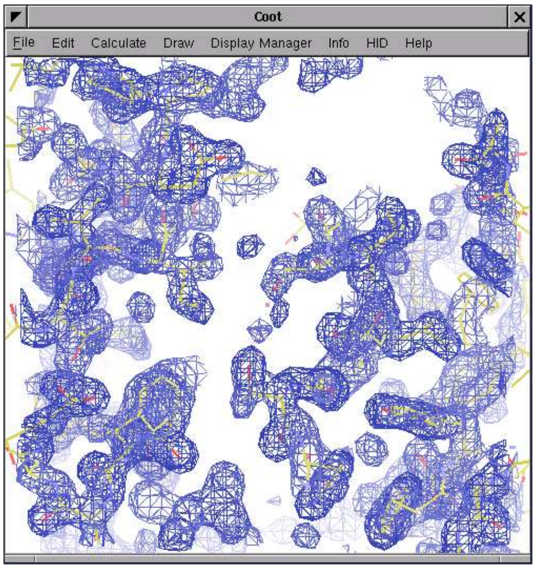

Figure 4: Coot after reading an MTZ file and zoomed in.

2.7 Recentre on Different Atoms

- Select "Draw" from the Coot menu-bar
- Select "Go To Atom..."
  [Coot displays the Go To Atom window]
- Select "1 A ASP" in the residue list
- Click "Apply" in the Go To Atom window
- At your leisure, use "Next Residue" and "Previous Residue" (or "Space" and "Shift" "Space"in the graphics window) to move along the chain.
- Click Middle-mouse over an atom in the graphics window
  [Coot recentres on that atom]
- Ctrl Left-mouse & Drag moves the view around. If this is a too slow and jerky:
  - Select Draw from Coot's menu-bar
  - Select the "Dragged Map..." menu item
  - Select "No" in the "Active Map on Dragging" window
  - Click "OK" in the "Active Map on Dragging" window

  Now the map is recontoured at the end of the drag, not at each step[3].

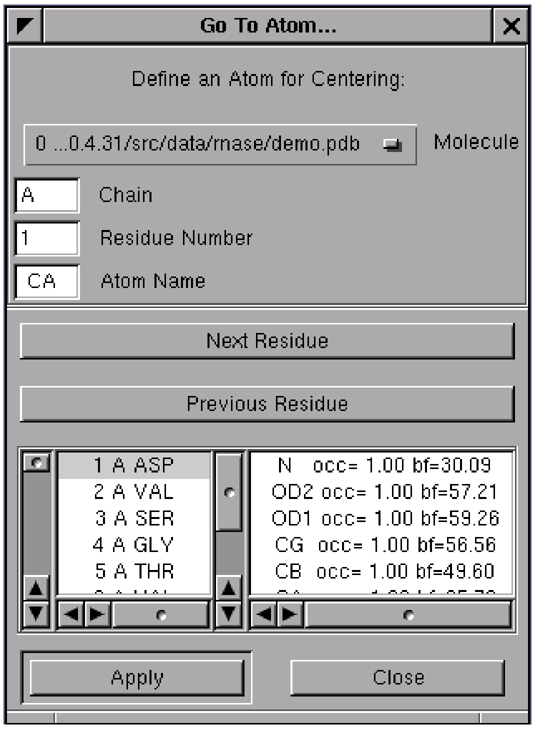

Figure 5: Coot's Go To Atom Window.

[3] which looks less good on faster computers.

- You can display the contacts too, as you do this:
  - Select "Measures" from the Coot menu-bar
  - Select "Environment Distances…"
  - Click on the "Show Residue Environment?" check-button
    - Also Click "Label Atom?" if you wish the Cα atoms of the residues to be labelled.
  - Click "OK" in the Environment Distances window
  - Click "Apply" in the Go To Atom window

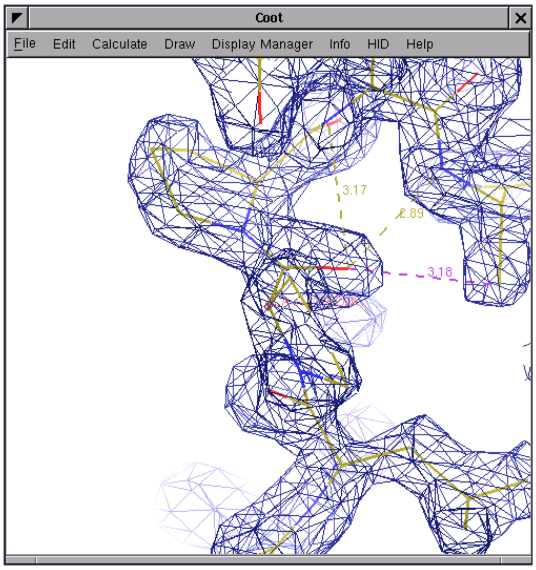

Figure 6: Coot showing Atom Label and environment distances.

You can turn off the Environment distances if you like.

2.8 Change the Clipping (Slab)

- Select "Draw" from the Coot menu-bar
- Select "Clipping…" from the sub-menu
  [Coot displays a Clipping window]
- Adjust the slider to the clipping of your choice
- Click "OK" in the Clipping window

Alternatively, you can use "D" and "F"[4] on the keyboard, or Control Right-mouse up/down (Control Right-mouse left/right does z-translation).

[4]think: Depth of Field.

2.9 Recontour the Map

- Scroll your scroll-wheel forwards one click[5]

  [Coot recontours the map using a 0.05electron/$Å^3$ higher contour level]

- Scroll your scroll-wheel forwards and backwards more clicks and see the contour level changing.

- If you don't have a wheel on your mouse you can use "+" and "-" on the keyboard.

- Note that the "Scroll" button in the Display Manager allows you to select which map is affected by this[6].

  [This button is not yet available in WinCoot]

2.10 Change the Map Colour

- Select "Edit" from the Coot menu-bar
- Select "Map Colour" in the sub-menu
- Select "0 xxx FWT PHWT" in the sub-menu

  [Coot displays a Map Colour Selection window]

- Choose a new colour by clicking on the colour widgets

  [Coot changes the map colour to match the selection]

- Click "OK" in the Map Colour Selection window

2.11 Select a Map

Select a map for model building:

Menubar: Calculate → Model/Fit/Refine...
[Coot displays the Model/Fit/Refine window]

Select "Select Map. . . " from the Model/Fit/Refine window

click OK (you want to select the map with ". . . FWT PHWT")

3 Model Building

"So what's wrong with this structure?" you might ask.

There are several ways to analyse structural problems and some of them are available in Coot.

Validate → Geometry Analysis → tutorial.pdb

[Note "Geometry Analysis" is under the "[Experimental]" sub-menu in WinCoot.]

[Coot displays a bar graph]

Look at the graph. The bigger and redder the bar the worse the geometry. There are 2 area of outstanding badness in the A chain, around 41A and 89A.

Let's look at 89A first - click on the block for 89A.

[Coot moves the view so that 89A CA is at the centre of the screen]

[5] don't click it *down*.

[6] by default it is the last map, which is not necessarily the map that you want.

3.1 Rotamers

- Examine the situation. . .
- The sidechain is pointing the wrong way. Let's Fix it...
- Menubar: Calculate → Model/Fit/Refine...

  [Coot displays the Model/Fit/Refine window]
- Select "Rotamers" from the Model/Fit/Refine window.
- In the graphics window, (left-mouse) click on an atom of residue 89A (the $C\gamma$, say)

  [Coot displays the "Select Rotamer" window]
- Choose the Rotamer that most closely puts the atoms into the lump of density
- Click "Accept" in the "Select Rotamer" window

  [Coot updates the coordinates to the selected rotamer]
- Click "RS Refine Zone" in the Model/Fit/Refine window.
- In the graphics window, click on an atom of residue 89A. Click it again.

  [Coot thinks for a while then displays the refined coordinates in white in the graphics and a new "Accept Refinement" window]
- Click "Accept" in the "Accept Refinement" window.

  [Coot updates the coordinates to the refined coordinates. 89A now fits the density nicely.]

  You can undo these operations by clicking on the "Undo" button (twice). You can then try again to fit the residue to the density using "Auto Fit Rotamer" or "Refine Zone" on that residue and click and drag the intermediate atoms into the density.

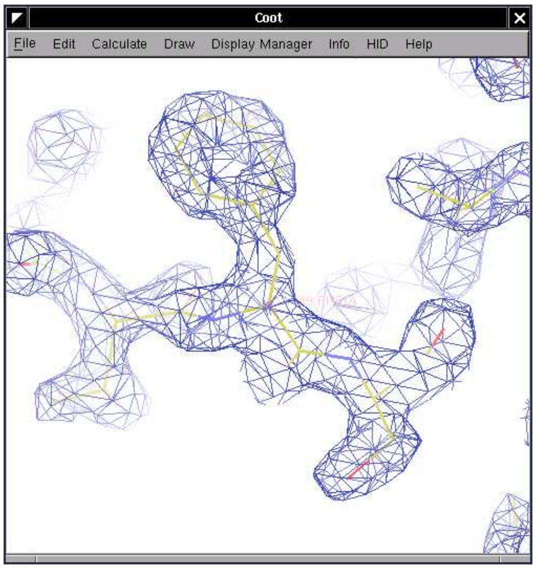

Figure 7: 89A now fits the density nicely.

3.2 More Real Space Refinement

Now let's have a look at the other region of outstanding badness:

- Click on the graph block for 41 A

  [Coot moves the view so that 41A CA is at the centre of the screen]

- Examine the situation. . .
- Residue 41 is in a mess and not fitting to the density. Can you fix it?
- Yes you can.
- The trick is to RS (Real Space) Refine that zone. So. . .
- You can either RS Refine a few residues (40, 41 and 42) or just 41. Take your pick.
- Click on "RS Refine Zone" in the Model/Fit/Refine window
- Select a range by clicking on atoms in the graphics window (either atoms in 40 then 42 or an atom in 41 twice)

  [Coot displays intermediate (white) atoms]

- Click and drag on some atoms until the atoms fit nicely in the density. If you want to move a *single atom* then *Ctrl Left-mouse* to select (just) it.

4 Blobology

4.1 Find Blobs

To be found under Validate (called "Unmodelled Blobs").

You can use the defaults in the subsequent pop-up. Press "Find Blobs" and wait a short while.

You will get a new window that tell you that it has found unexplained blobs. Time to find out what they are.

4.1.1 Blob 3

Let's start from Blob 3

- Click on "Blob 3"

  [Coot centres the screen on a blob]

- Examine the situation. . .
- We need something tetrahedral there. . .
- "Place Atom At Pointer" on the Model/Fit/Refine window

  [Coot shows a Pointer Atom Type window]

- "SO4" in the new window. . .
- In the "Pointer Atom Added to Molecule:" frame, change "New Molecule" to "tutorial.pdb"
- Click OK.

- Examine the situation…
- the orientation is not quite right.
- Let's RS Refine it (you should know what to do by now…)
- ("RS Refine Zone" then click an atom in the SO_4 twice. Accept)
  [The SO_4 fits better now].

4.1.2 Blob 2

Click on 'the button 'Blob 2" and examine the density. Something is missing from the model. What?

This protein and has been co-crystallized with its ligand substrate. That's what missing: 3' GMP. So let's add it…

- File → Get Monomer…
- Type "3GP" in the box (Use Upper Case). Press return…
  [Coot pauses for a few seconds while LIBCHECK and REFMAC run]
  [3GP appears]
  Note: In WinCoot "File → Get Monomer…" won't work. Instead open the file `monomer-3GP.pdb`.
  It is generally easier to work without hydrogens, so let's delete them
- "Delete…" from the Model/Fit/Refine window
- "Hydrogens in Residue"
- click on an atom in 3GP
  [Hydrogens disappear]
  The 3GP is displaced from where we want it to be.
- "Rotate/Translate Zone" from the Model/Fit/Refine window
- click on an atom in 3GP twice
  [Intermediate (white) atoms appear]
- Drag it or use the sliders to move the intermediate fragment to the approximately the right place.
- Click "OK" in the slider window
- Now optimize the fit using "RS Refine Zone" like we did before.
  Now merge this ligand into the protein:
- Calculate → Merge Molecules…
- Click (activate) "monomer-3GP.pdb"
- into Molecule:
  tutorial.pdb
- Merge

This is a far as I expect you to get.

4.2 Make a (Pretty?) Picture

(The pretty picture doesn't work with WinCoot yet):

- Arrange a nice view
- Press F8
- Wait a few seconds...

5 Extra Fun (if you have time)

5.1 Waters

Fit waters to the structure

- "Find Waters..." in the Model/Fit/Refine window, then OK (using the defaults)

  [Waters appear]

To check the waters:

- Click on "Measures" in the main menubar.
- Click on Environment Distances
- Click on the "Show Residue Environment?" check-button
- Change the distances as you like
- Use the Go To Atom widget to go to the first water (probably towards the end of the list of residues in Chain "W")
- Use the Spacebar to navigate to the next residue (and Shift Spacebar to go backwards)
- If you don't like the fast sliding[7] you can turn it off:
  - Click "Draw" in the main menubar
  - Click "Smooth Recentering..."
  - Click "No"

5.2 Add Terminal Residue

[To be found on the Model/Fit/Refine dialog]

Use the Go To Atom window to go to residue 93 A. Notice there is some density unaccounted for - it is the as yet unbuilt C terminus of the A chain. This is Blob 1.

Add Terminal Residue and click on 93 A CA (or any atom in that residue).
[Coot displays intermediate (white) atoms]

Accept them.

Now autozone refine them:

Click on "Refine Zone" in the Model/Fit/Refine dialog

[7] it's not very good for water checking

Press the "A" key on the keyboard[8]

While pressing A click on an atom in the new residue.
[Coot displays intermediate atoms]

Accept them

Do the same for the next 2 residues (add and autozone refine).

You should now have 3 extra ALAs at the C-terminus.

Mutate them (and fit them):

Click on "Mutate Molecule..." in the "Calculate" menu of the main menubar.

The new residue numbers are 94 to 96 inclusive.

Choose the sequence `QTC`

Click on the "Autofit the mutated residues" check button.

Click on the "Mutate" button

5.3 Mutate Residue

You can also mutate residues individually:

Use "Simple Mutate" on the Model/Fit/Refine dialog. Click on an atom in the residue that you wish to mutate.

You can choose the rotamer manually using "Rotamers" in the Model/Fit/Refine dialog.

5.4 Display Symmetry Atoms

To be found on "Draw" → "Cell & Symmetry" menu item in the main menu.

Try:

Show Symmetry Atoms? → Yes

Symmetry as Calphas? [on]

Colour symmetry by molecule [on]

Symmetry Radius 50Å

Colour Merge → 0.1

Show Unit Cell? [on]

OK

Zoom out and have a look at how the molecules pack together.

5.5 Refine with Refmac

This option does not work on WinCoot.

Refmac is the program that does Maximum Likelihood refinement of the model and the interface to it can be found on the Model/Fit/Refine dialog.

First you need to read in an MTZ file (as you did previously) but this time assign the Refmac column labels[9] using "File" → "Open MTZ, CIF or phs…"

Now click on "Run Refmac…" in the Model/Fit/Refine window

The defaults should be fine...

"Run Refmac" in the new window…

…Wait…

[The cycle continues…]

[8] "A" for Autozone

[9] Which are the default labels, in this case.

Colophon

This document is written using XEmacs 21.5 in LaTeX using AUCTeX and is distributed with the Coot source code.