Sparky

NMR Assignment and Integration Software

NMR software

- Paid
  - TopSpin
    - Developed and mainly used for Bruker spectrometers controlling
    - Many functions for processing, view and analysis of one- and multi-dimensional spectra
    - Basically impossible analysis of multi-dimensional
  - Mnova
    - Processing, analysis and visualization of 1D and 2D spectra
    - Support of different formats of spectra (Bruker, Jeol, Agilent...)
    - Free trial version
  - ACD/NMR processor
    - see Mnova
    - Free version for academic purposes with noncomplete functionalities

NMR software

- Freeware
  - NMRPipe
    - Multiplatform
    - Processing and analysis of any spectra
    - Based on UNIX systems → knowledge of UNIX environment is advantage
  - SpinWorks
    - For the Windows systems (emulator of Windows are needed for UNIX a Mac)
    - Processing and analysis of 1D and 2D spectra
    - User very unfriendly
  - **Sparky**

Sparky

NMR Assignment and Integration Software

Originally developed on University of California, San Francisco, currently upgraded on University of Wisconsin–Madison (as **NMRFAM-Sparky**)

- Multiplatform (Windows and Linux OS)
- Free for download:

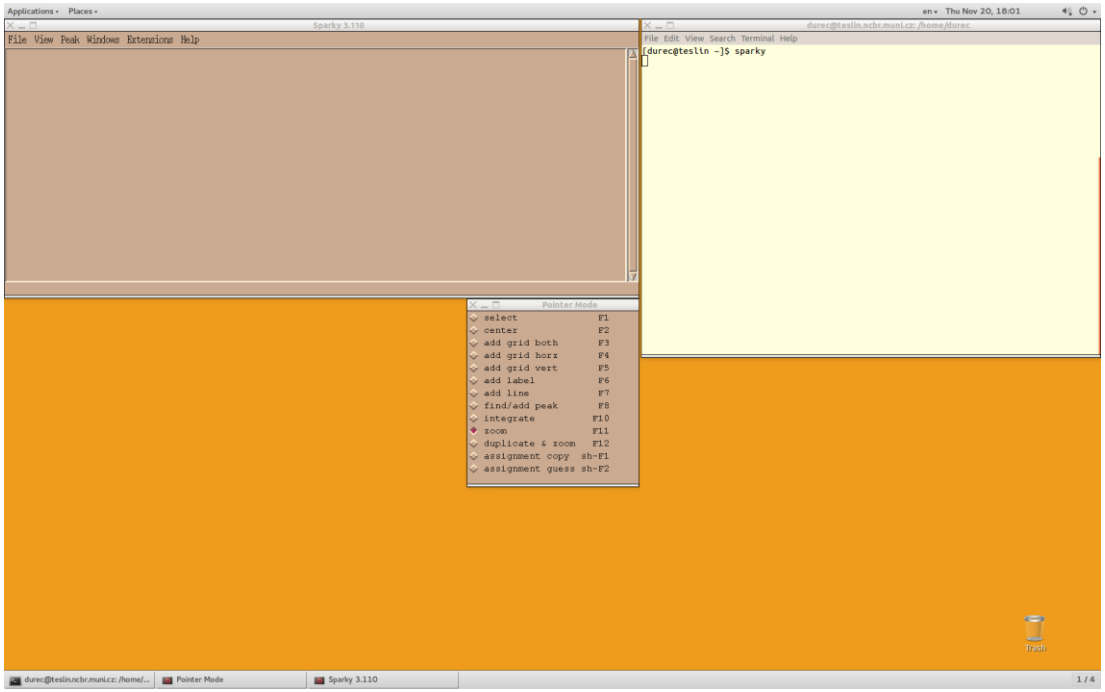
  https://nmrfam.wisc.edu/nmrfam-sparky-distribution/
- Written in Python language, open source, it is not developed anymore (last version is from the 2008)
- Fast and undemanding for hardware
- **It can handle only multi-dimensional spectra (2D, 3D)**

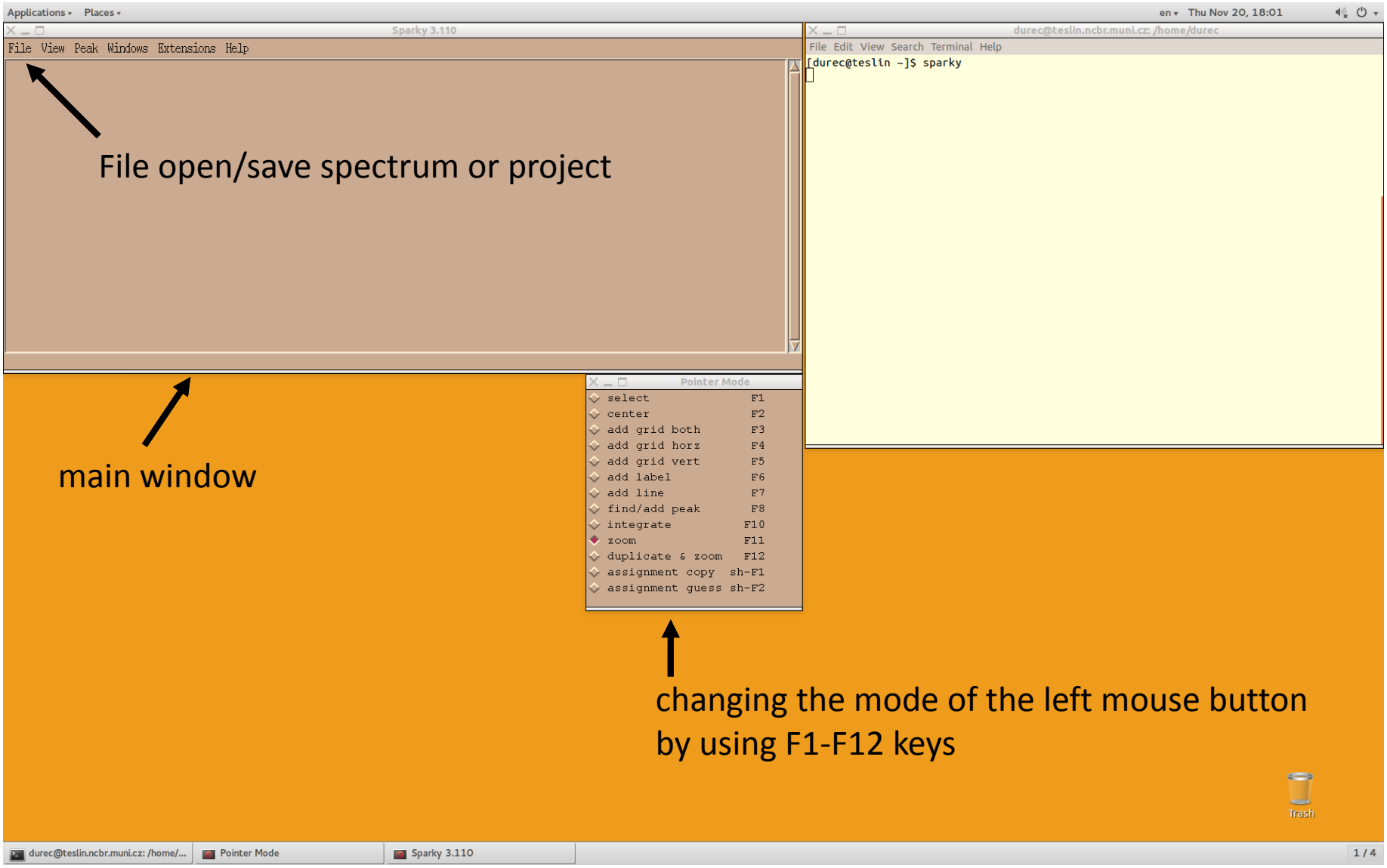
Sparky - spectra

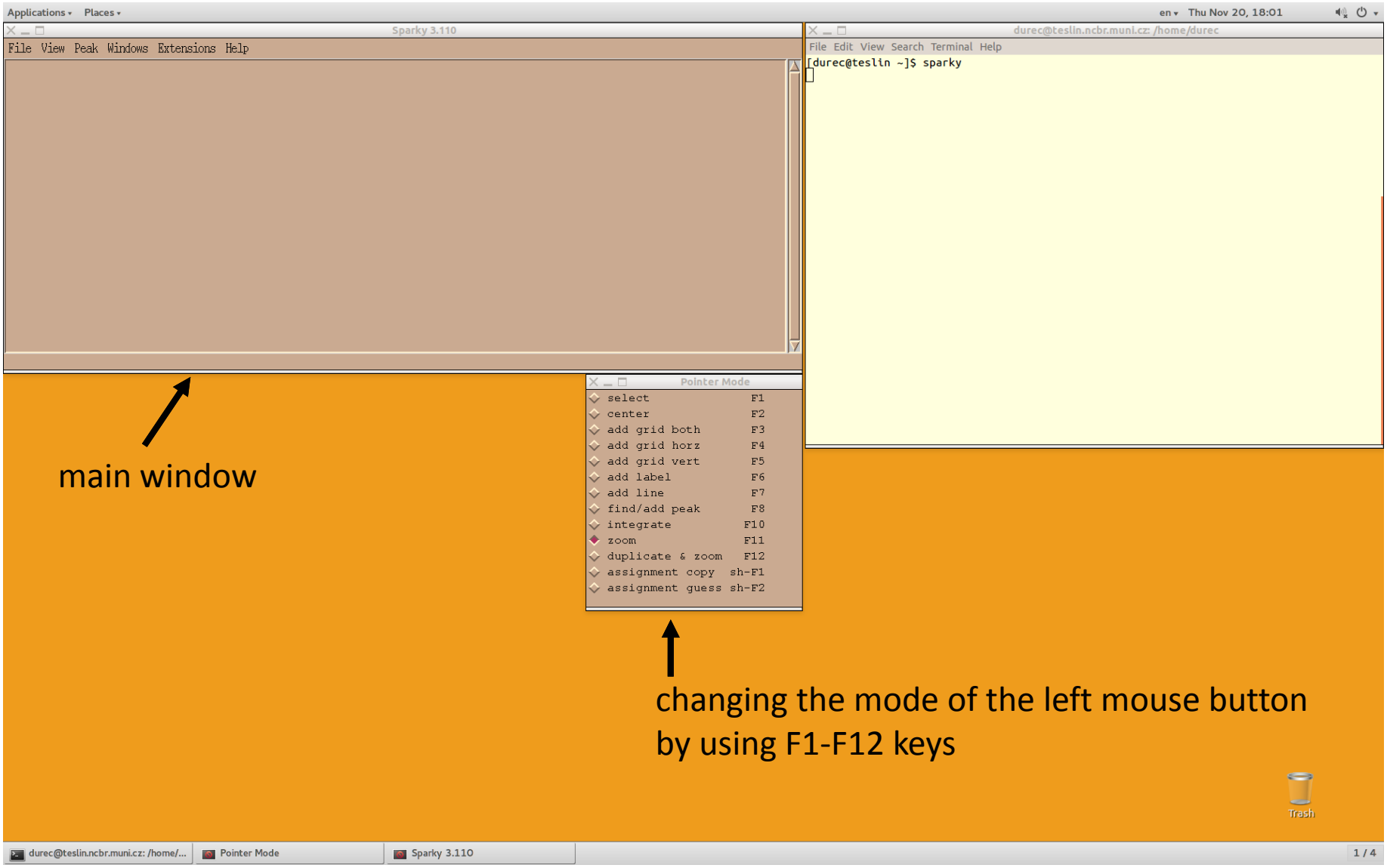
- It can work with different formats of spectra, but to work with them is hard and slow (computationaly)
  - Conversion of spectra into *.ucsf format
    - Unix: bruk2ucsf 2rr *name_of_the_spectrum*.ucsf
    - Windows: on the command line bruk2ucsf.exe 2rr *name_of_the_spectrum*.ucsf
  - Spectrum does not contain any information about acquisition, temperature and so on → it is necessary to give the *.uscf file proper name (colchicine_noesy_800_D2O_25C.ucsf)
- It has its own structure of folders
  - Lists, Projects, Save
- Sparky start
  - Windows: /sparky/bin/sparky.bat

Sparky - start

- Windows: batch file – sparky.bat




Applications
Places
en
Thu Nov 20, 18:01
Sparky 3.110
File View Peak Windows Extensions Help
File open/save spectrum or project
durec@teslin.ncbr.muni.cz: /home/durec
File Edit View Search Terminal Help
[durec@teslin ~]$ sparky
main window
Pointer Mode
select F1
center F2
add grid both F3
add grid horz F4
add grid vert F5
add label F6
add line F7
find/add peak F8
integrate F10
zoom F11
duplicate & zoom F12
assignment copy sh-F1
assignment guess sh-F2
changing the mode of the left mouse button
by using F1-F12 keys
Trash
durec@teslin.ncbr.muni.cz: /home/...
Pointer Mode
Sparky 3.110
1 / 4


Applications
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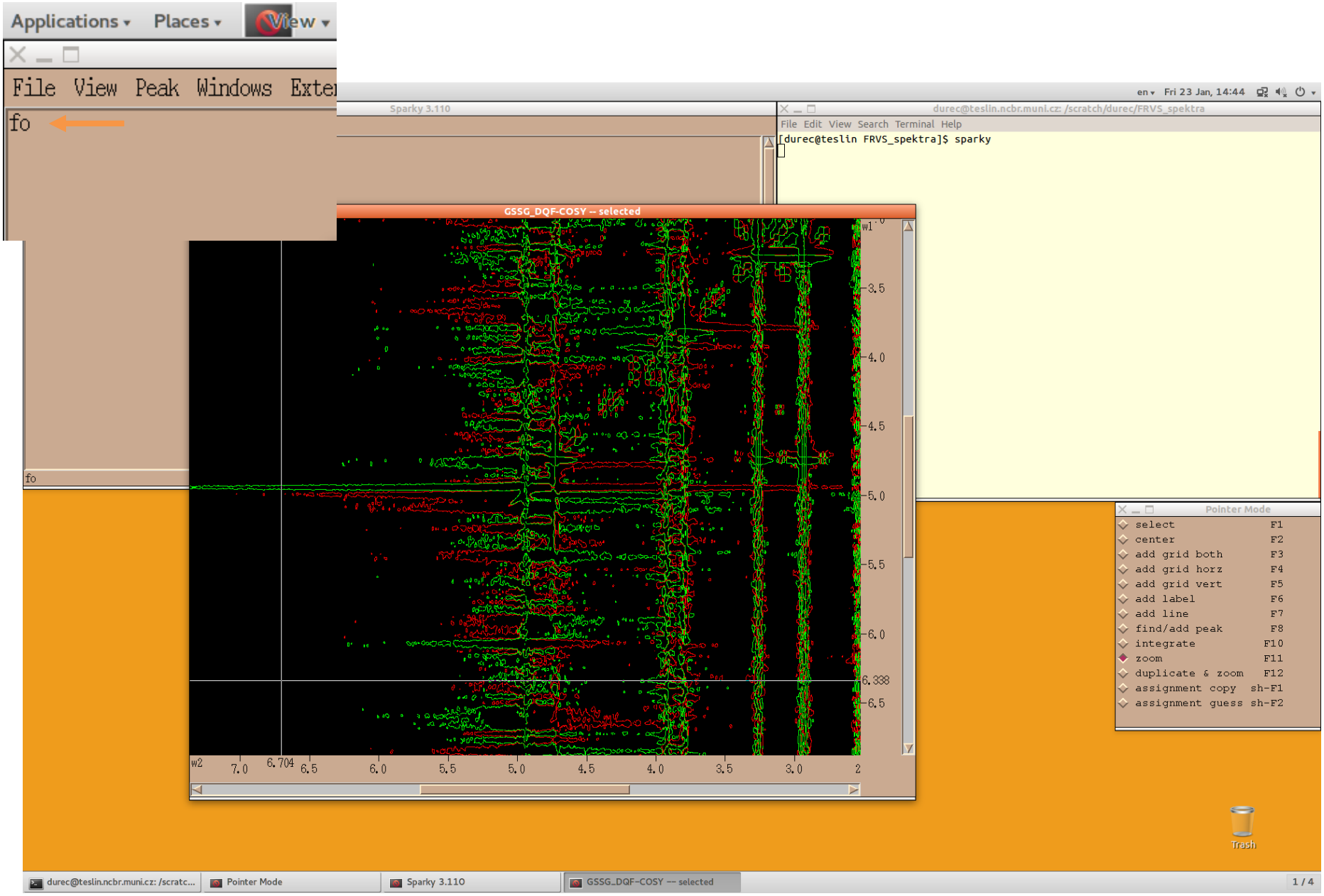
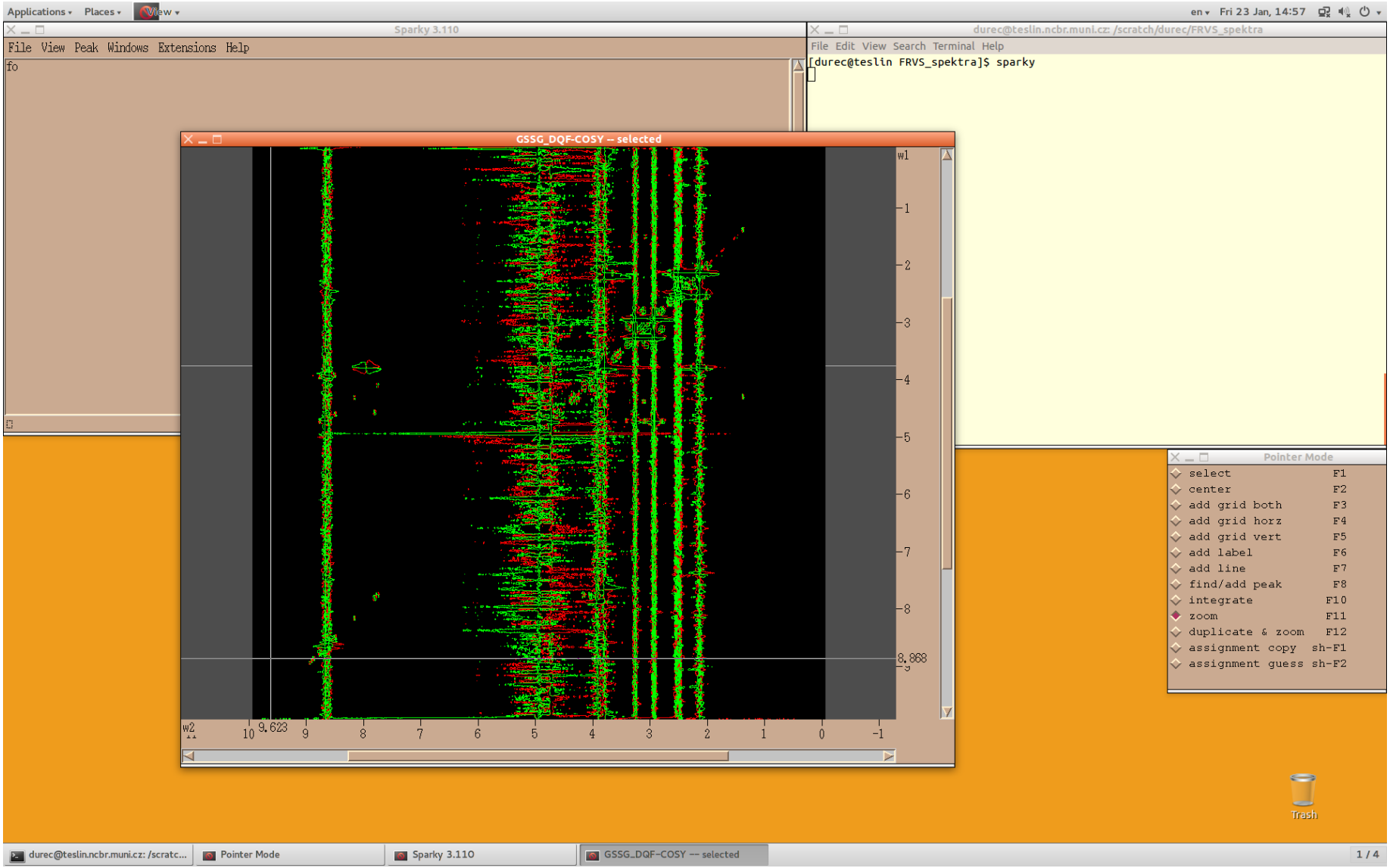
main window
changing the mode of the left mouse button
by using F1-F12 keys
Trash
durec@teslin.ncbr.muni.cz: /home/...
Pointer Mode
Sparky 3.110
1 / 4

Sparky - control

- Control by using
  - Two letter shortcuts (case-sensitive)
  - Menu in the main window
- *.ucsf file - spectral data from a NMR exp
- *.save file – Sparky format (plain text) contains all settings, assignment
- *.proj file – Sparky project file contains link to multiple *save files
- Open spectrum/project: ***fo*/*jo***
- Save spectrum/project as: ***fa*/*ja***
- Save spectrum/project: ***fs*/*js***
- Backup files*.bak are created automatically during the saving of the spectrum

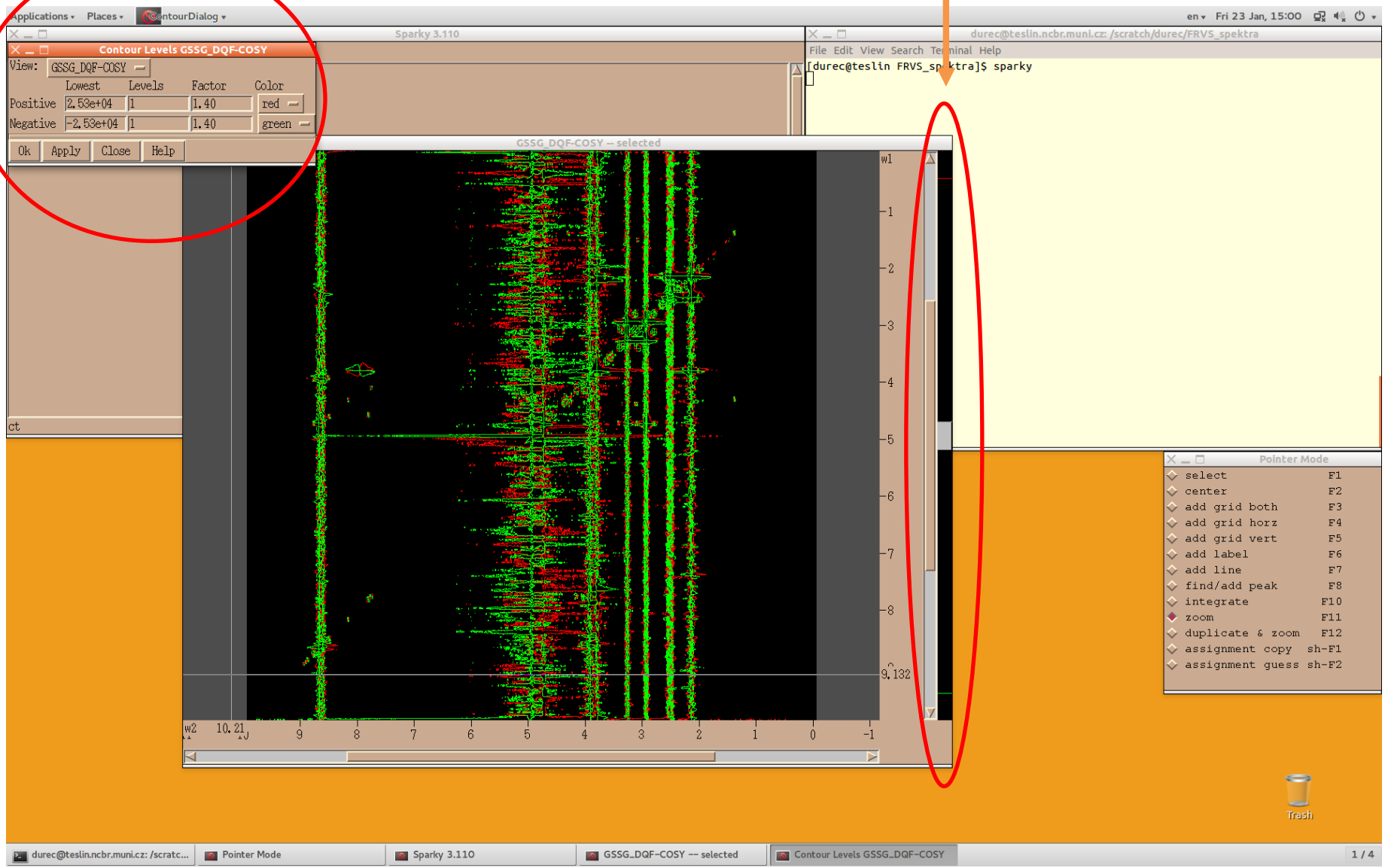

Applications
Places
View
File View Peak Windows Exte
fo
Sparky 3.110
durec@teslin.ncbr.muni.cz: /scratch/durec/FRVS_spektra
File Edit View Search Terminal Help
[durec@teslin FRVS_spektra]$ sparky
GSSG_DQF-COSY -- selected
w1
3.5
4.0
4.5
5.0
5.5
6.0
6.338
6.5
w2
7.0
6.704
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2
Pointer Mode
select F1
center F2
add grid both F3
add grid horz F4
add grid vert F5
add label F6
add line F7
find/add peak F8
integrate F10
zoom F11
duplicate & zoom F12
assignment copy sh-F1
assignment guess sh-F2
Trash
durec@teslin.ncbr.muni.cz: /scratc...
Pointer Mode
Sparky 3.110
GSSG_DQF-COSY -- selected
1 / 4

zf – full view


Applications
Places
Fri 23 Jan, 14:57
Sparky 3.110
File View Peak Windows Extensions Help
fo
durec@teslin.ncbr.muni.cz: /scratch/durec/FRVS_spektra
File Edit View Search Terminal Help
[durec@teslin FRVS_spektra]$ sparky
GSSG_DQF-COSY -- selected
w1
w2
9.623
8.868
Pointer Mode
select F1
center F2
add grid both F3
add grid horz F4
add grid vert F5
add label F6
add line F7
find/add peak F8
integrate F10
zoom F11
duplicate & zoom F12
assignment copy sh-F1
assignment guess sh-F2
Trash
durec@teslin.ncbr.muni.cz: /scratc...
Pointer Mode
Sparky 3.110
GSSG_DQF-COSY -- selected
1 / 4

ct – contour levels

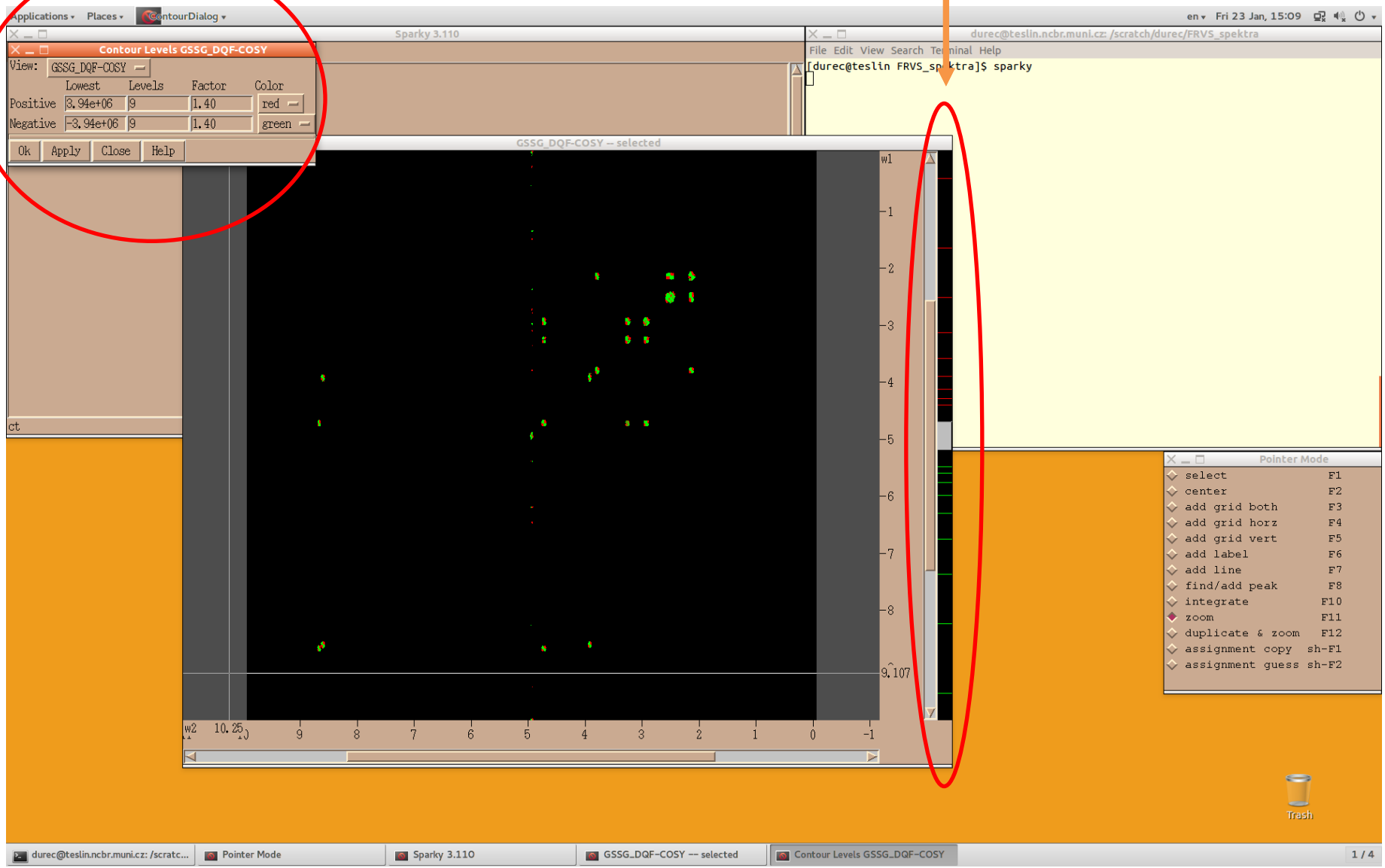
vC – contour scale



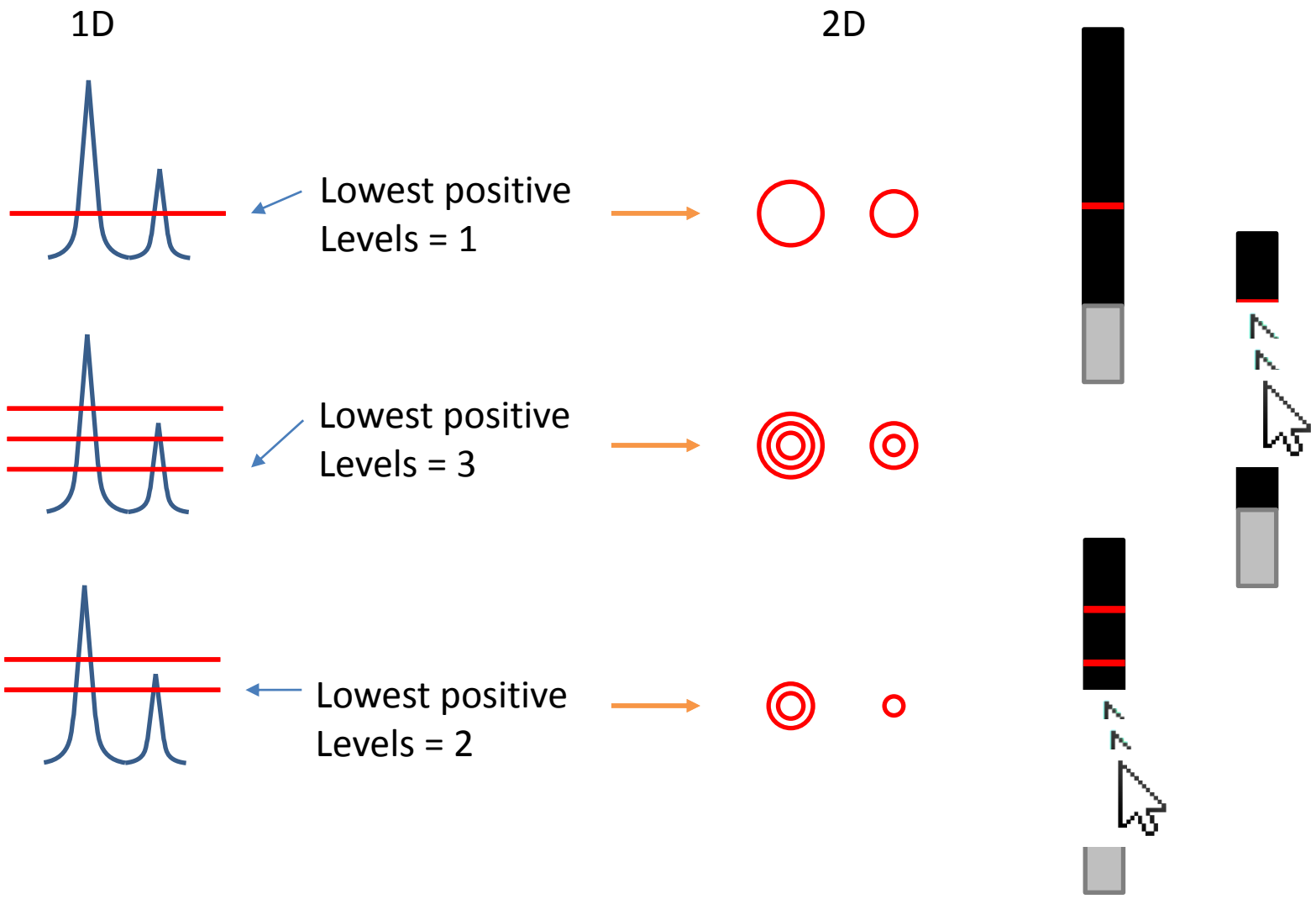
adjustment of the contours:

manually overwrite the values

change by dragging the left mouse button



adjustment of the contours:



zooming the spectrum:

keyboard shortcut: ***zi*** (zoom in)

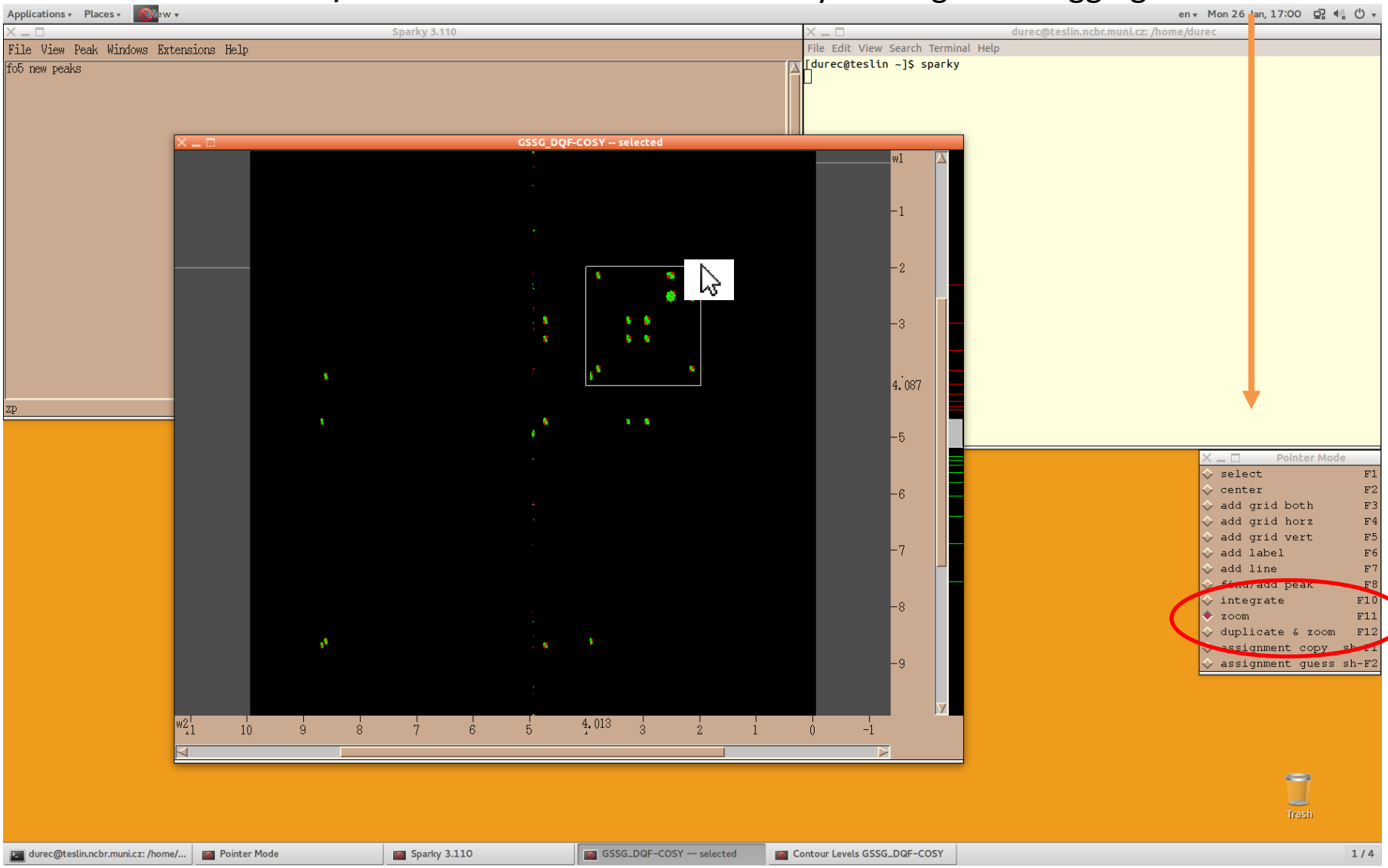
preset zoom in

alternatively use of middle mouse bottom

pointer mode– zoom: key F11

selected region is zoomed

by clicking and dragging of the mouse

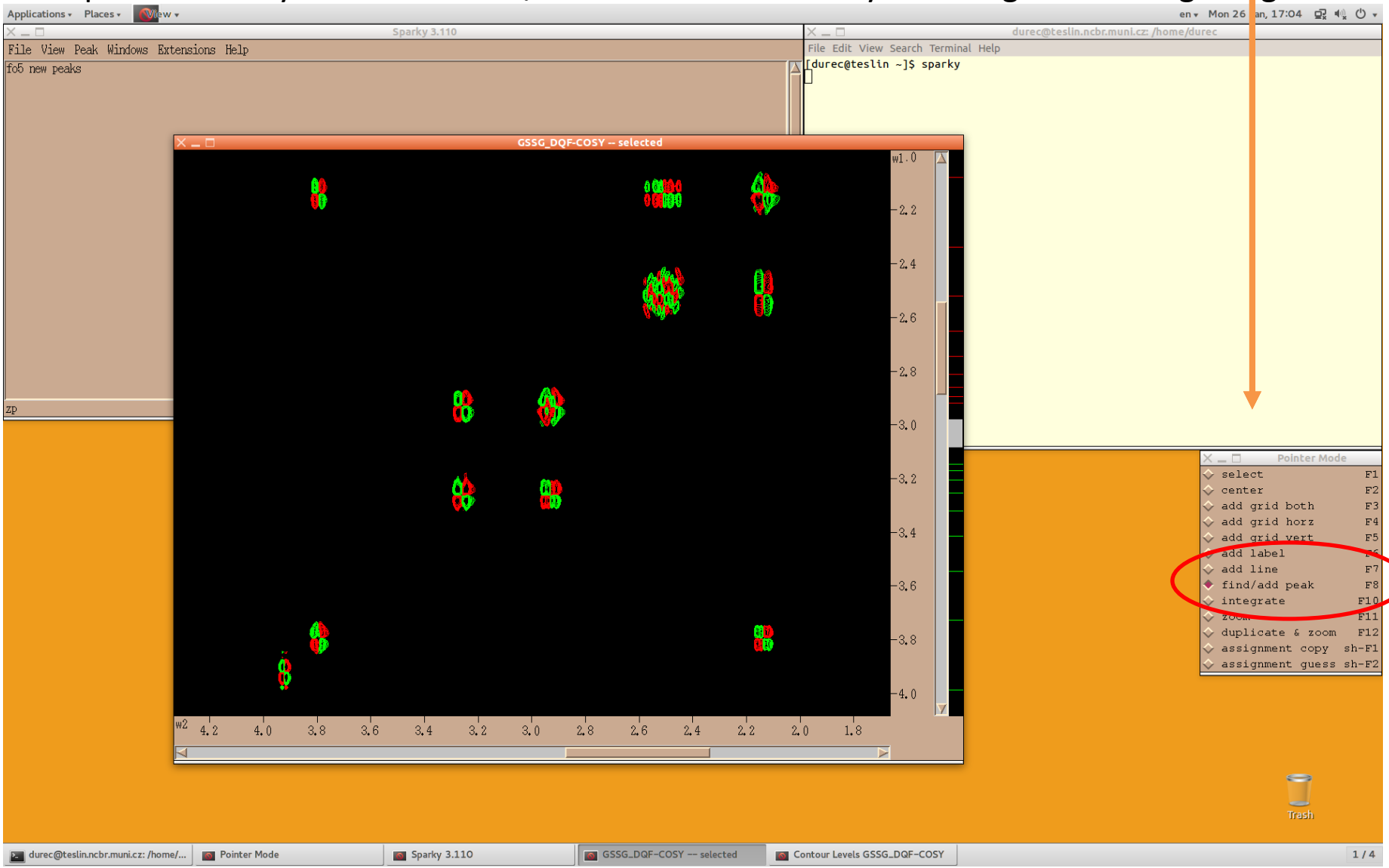


PEAK PICKING:

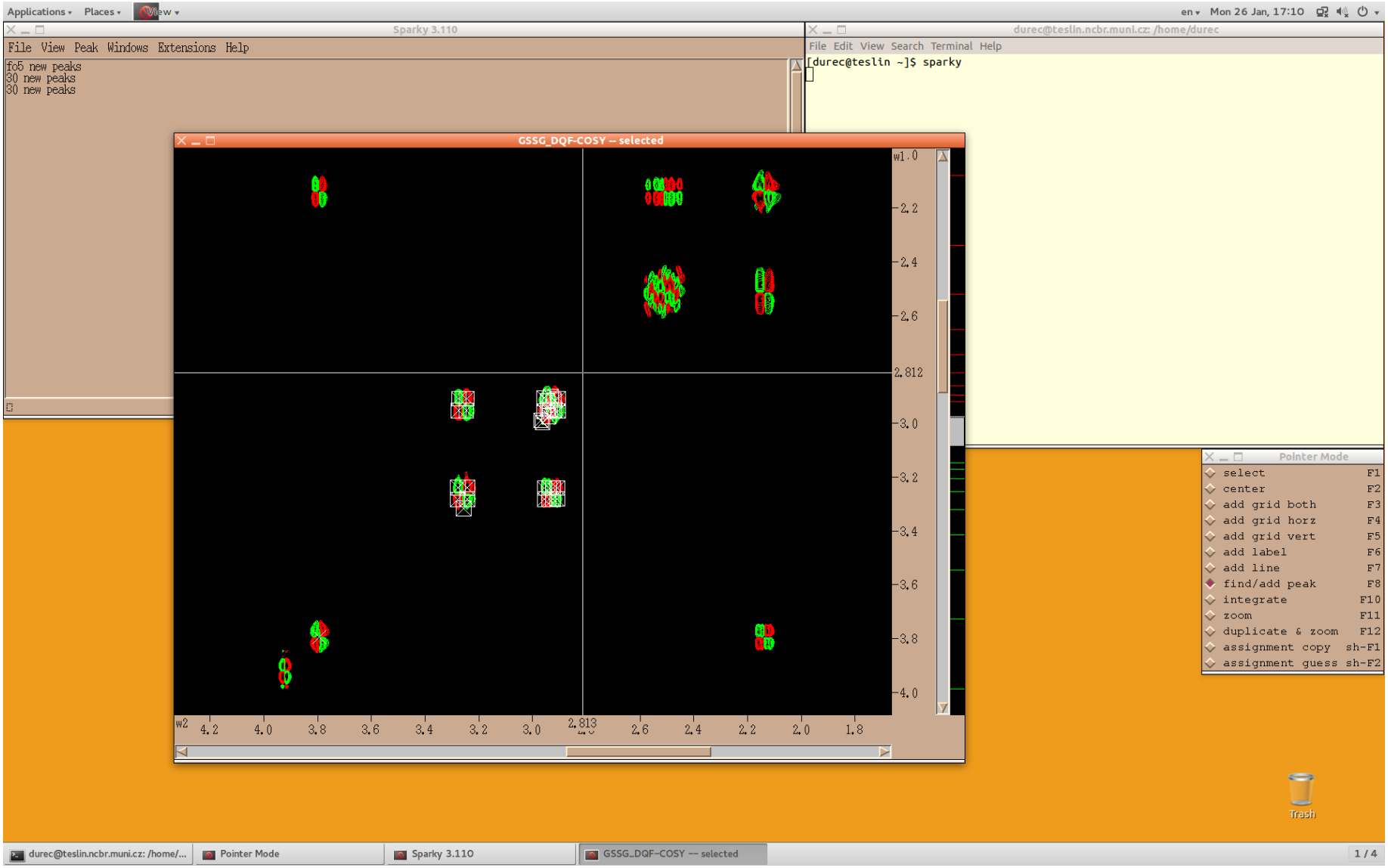
pointer mode – **find/add peak: key F8**

one peak is added by one left click,

peaks in every local maximum/minimum are added by selecting the rectangle region

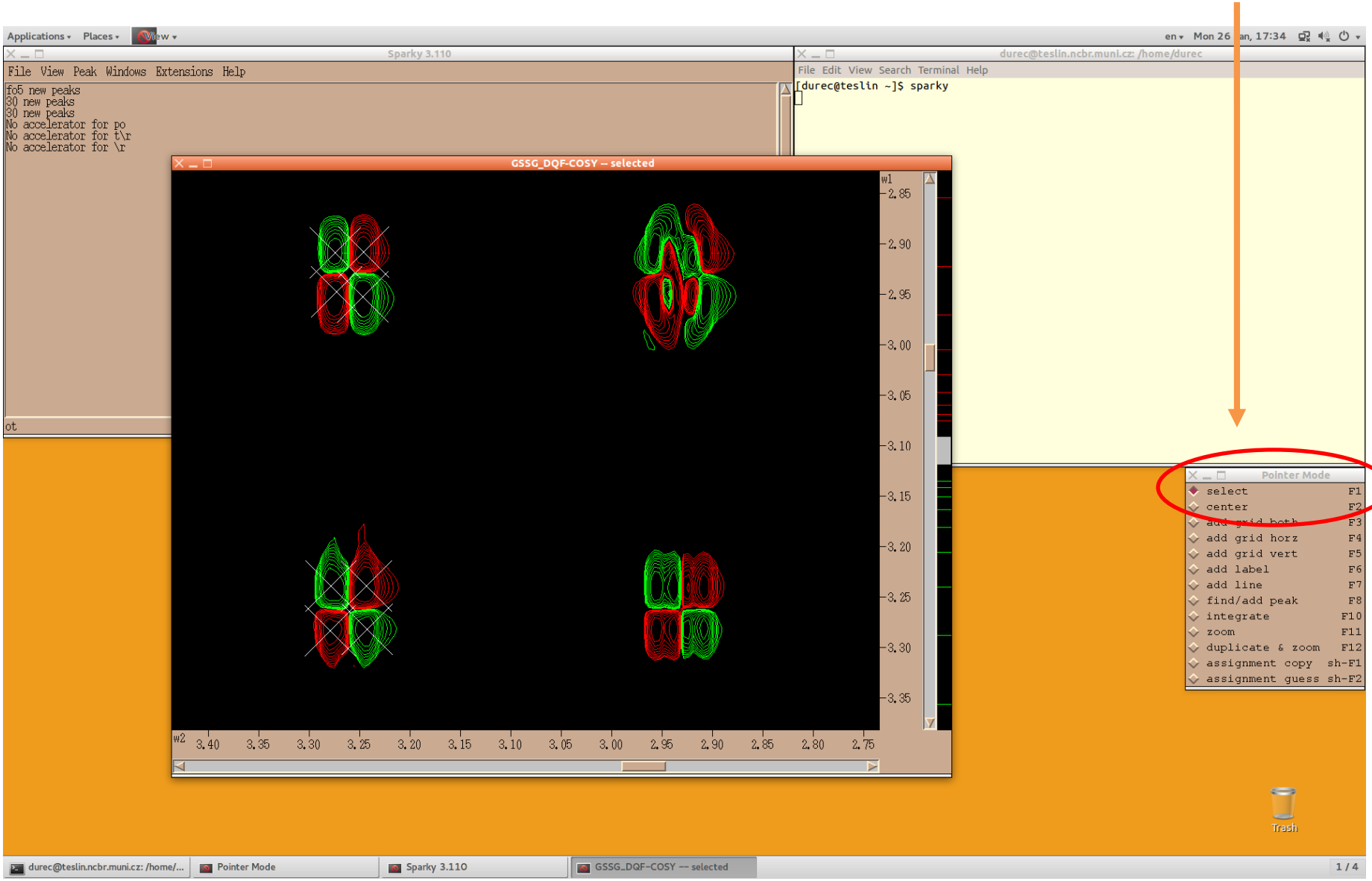


peak picking: changing the size of ornaments (peaks, labels...): ***ot*** → sizes → change the size as desirable (proper value 0.05) → apply

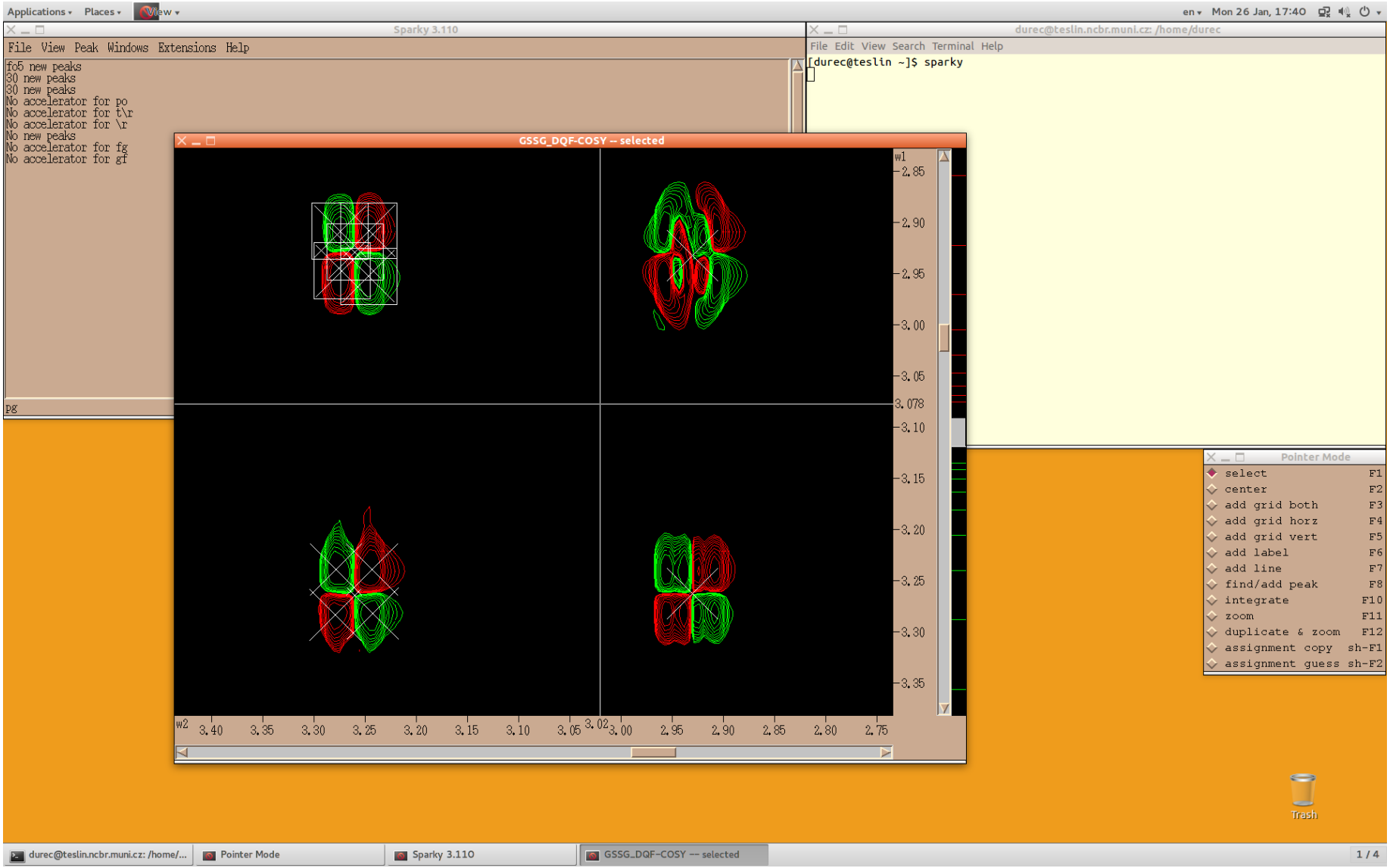


PEAK SELECTION

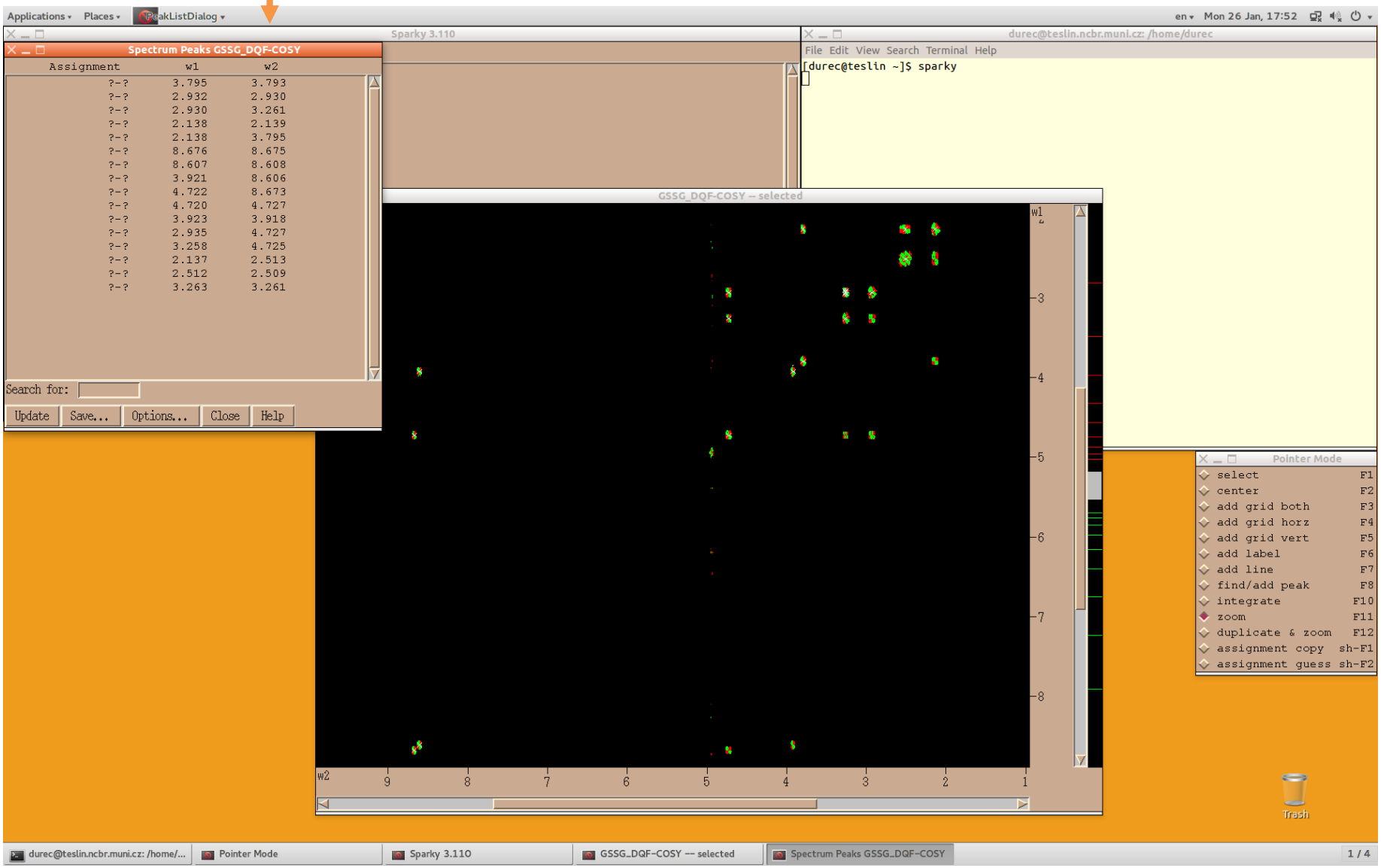
selection: pointer mode – **select: key F1**
one object is selected by clicking on it,
more object are selected by dragging the mouse (or Shift+click)



peak picking: adding of central peak from the selected group of peaks – ***pg***
(useful for multiplets in COSY spectra)

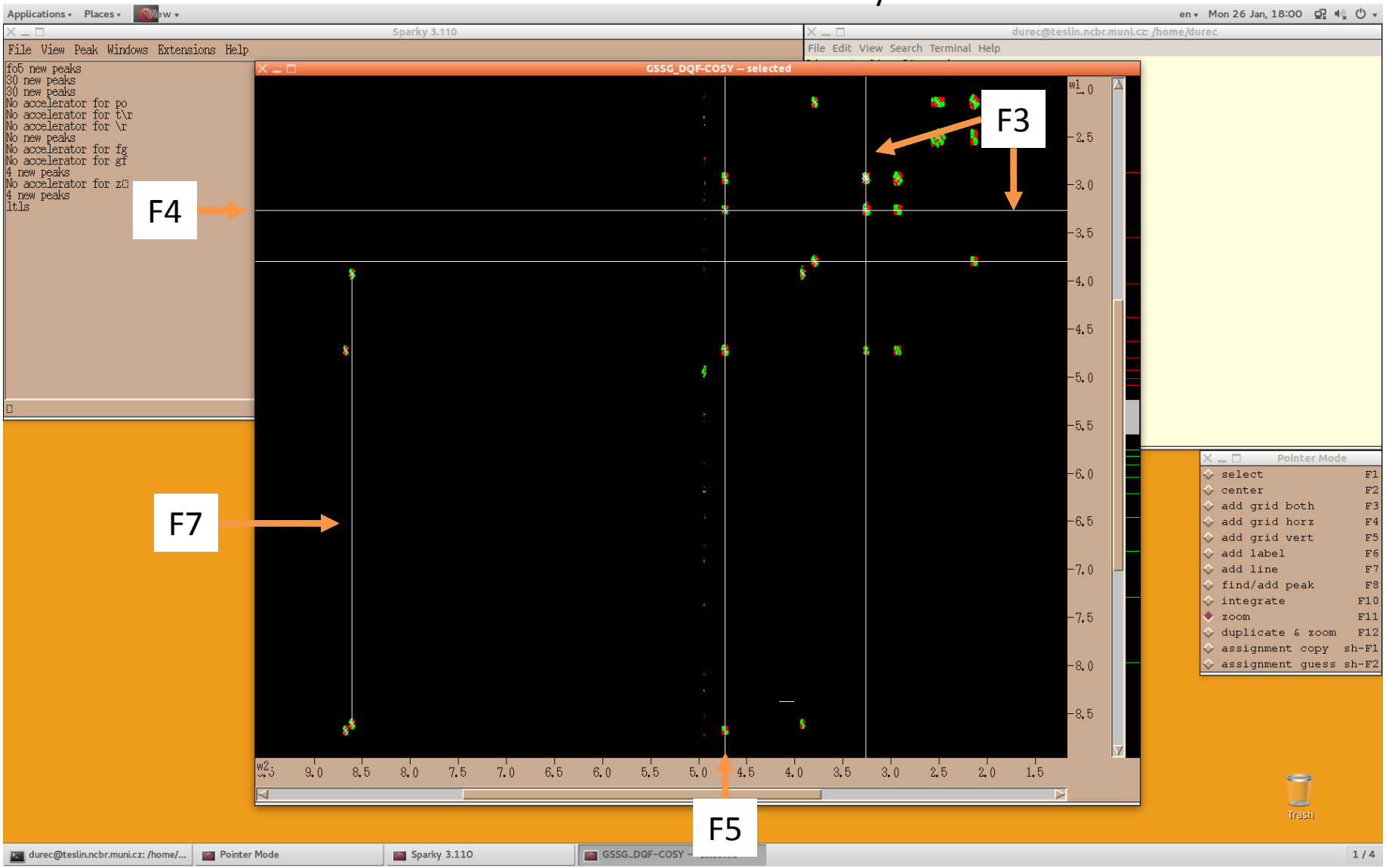


peak picking: list of the peaks – ***lt***



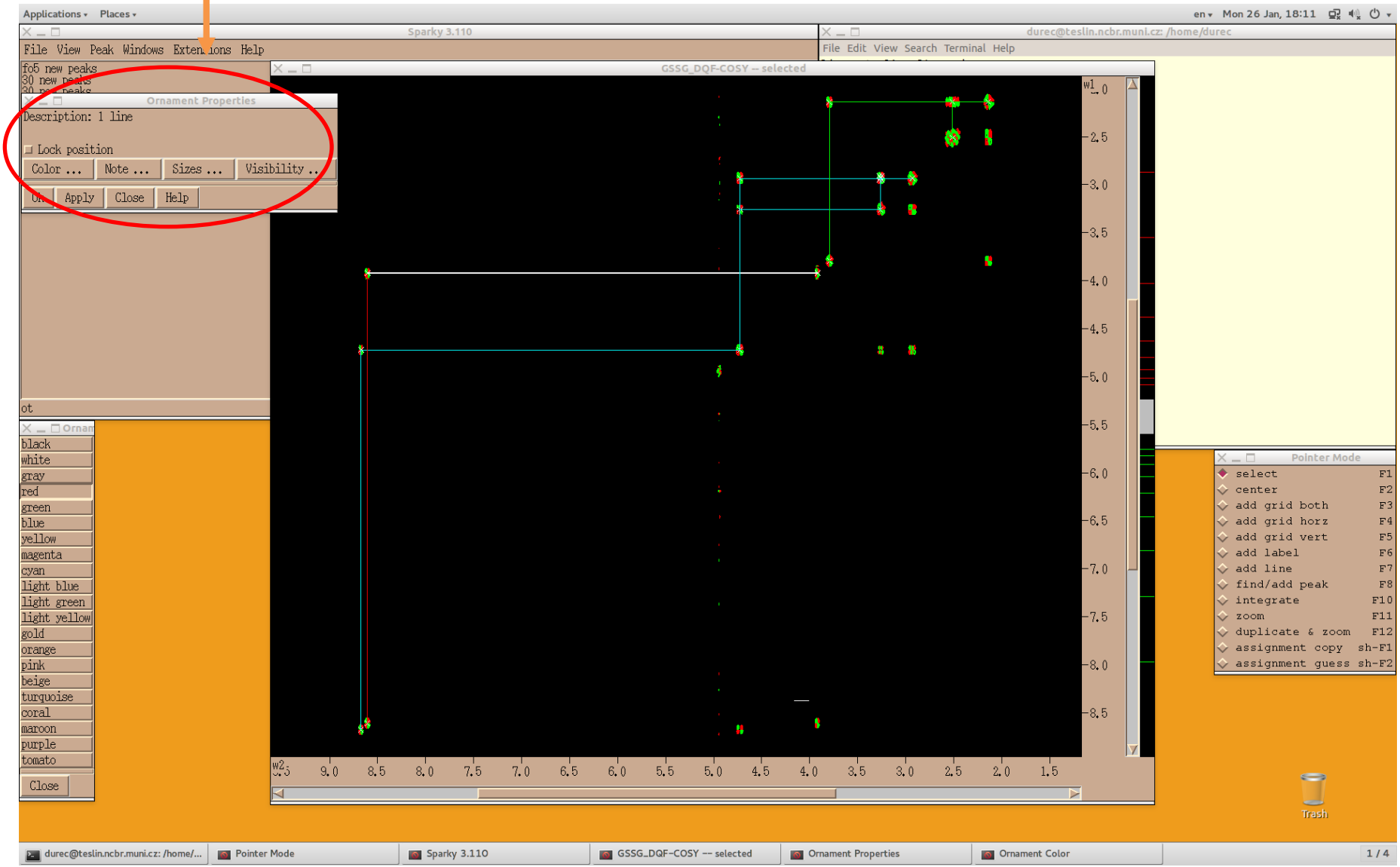
identification of the spin systems:

add vertical and horizontal line: key F3
add horizontal line: key F4
add vertical line: key F5
add a vector: key F7



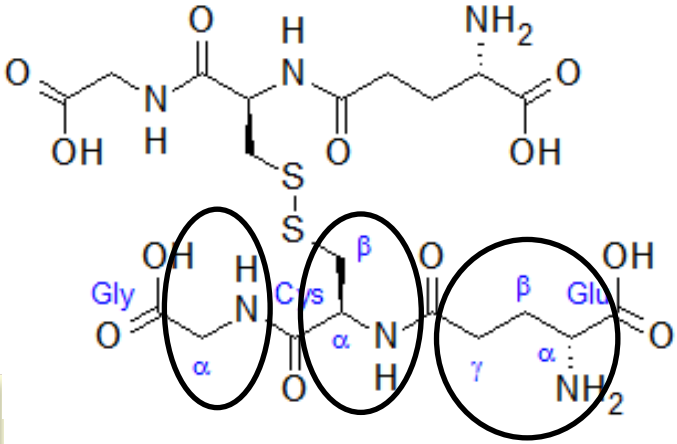
identification of the spin systems:

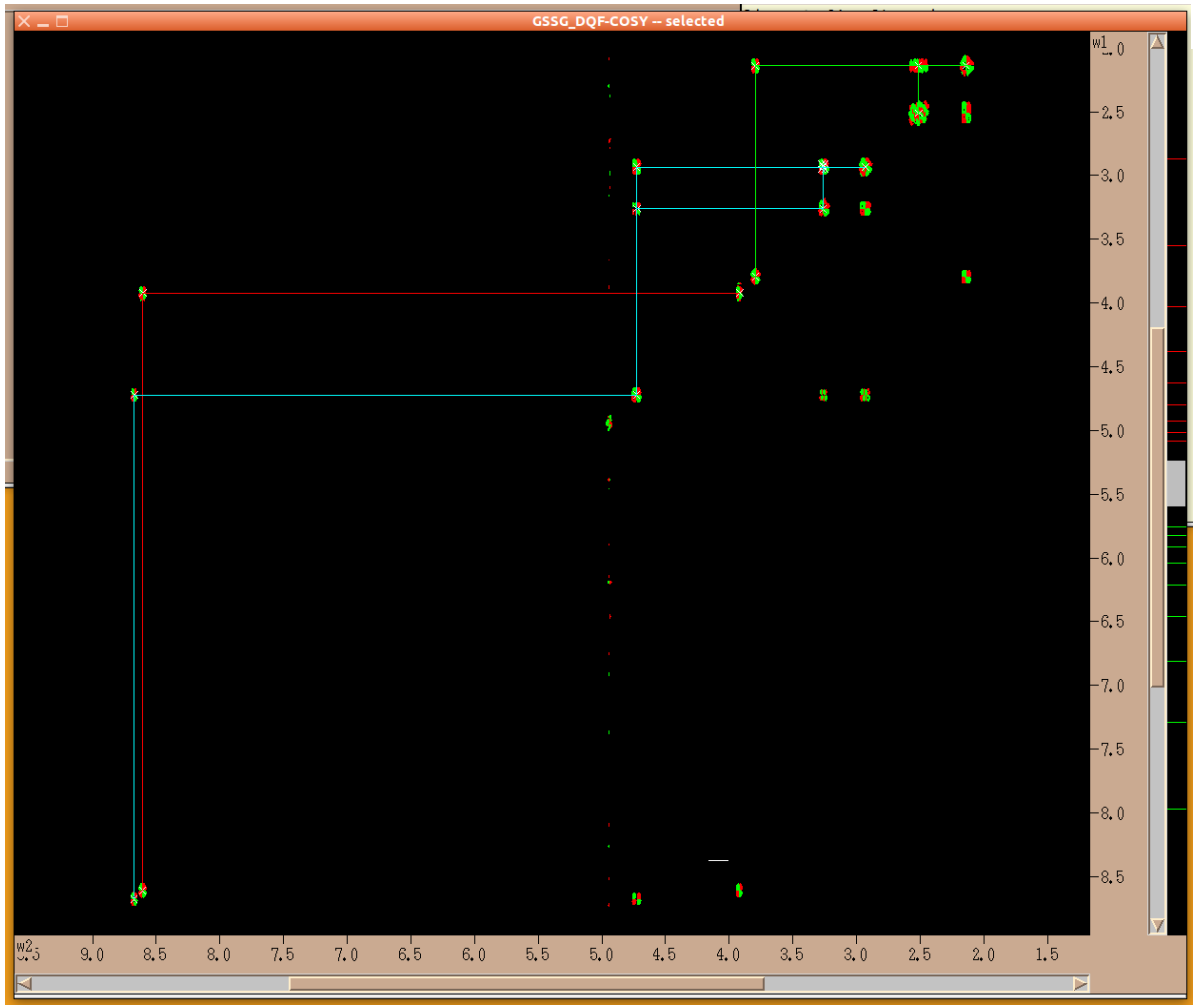
changing the color of ornaments (lines, labels...): ***ot*** → color → select object/s→ choose the color → apply



identification of the spin systems:

COSY – correlation through bonds, isolated spin systems





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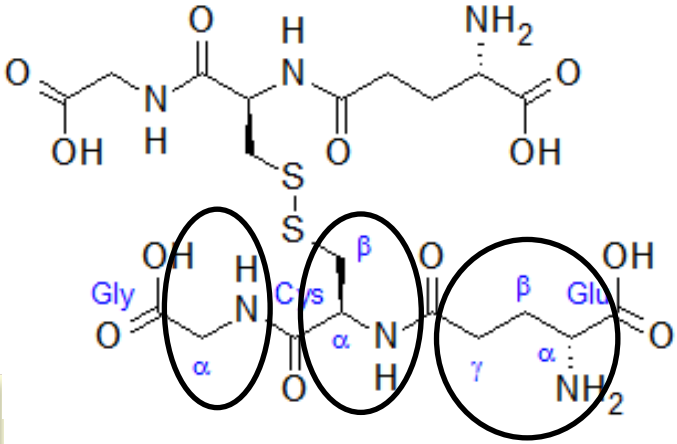
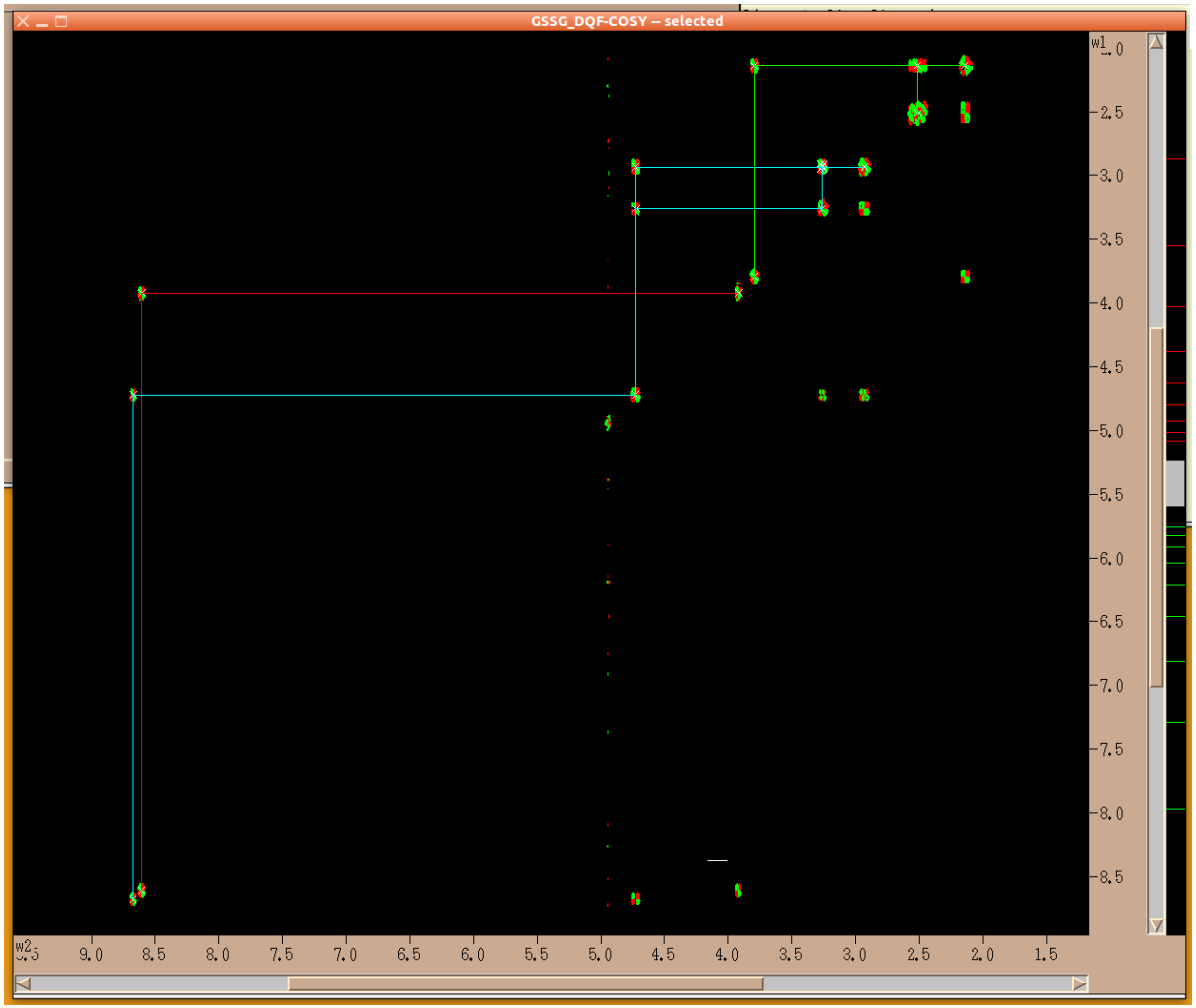
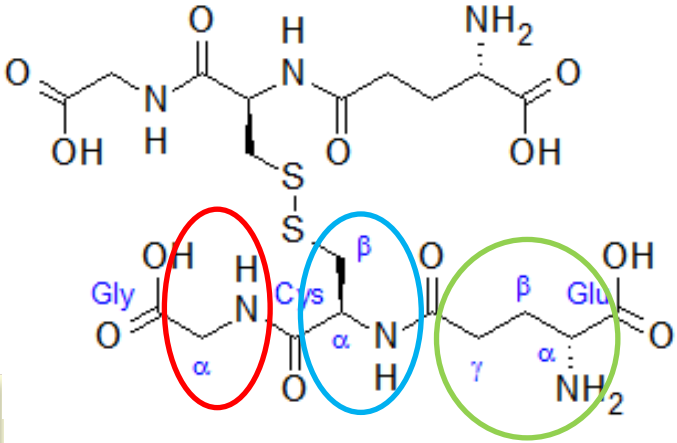


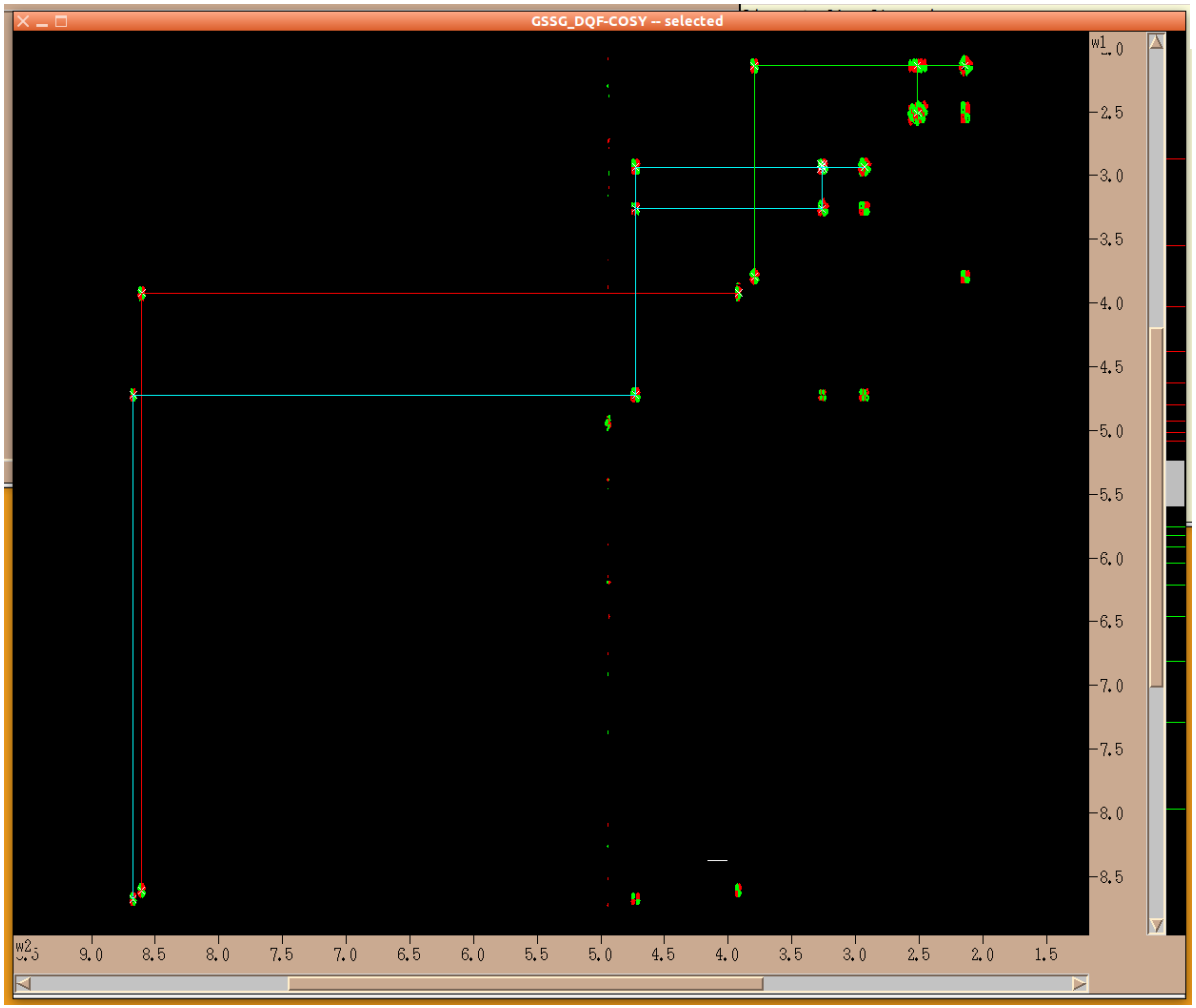


expected number of signals:
Gly – 2: Hα, NH
Cys – 4: Hα, NH, Hβ‘, Hβ‘‘
Glu – 3: Hα, Hβ, Hγ

identification of the spin systems:

COSY – correlation through bonds, isolated spin systems





expected number of signals:

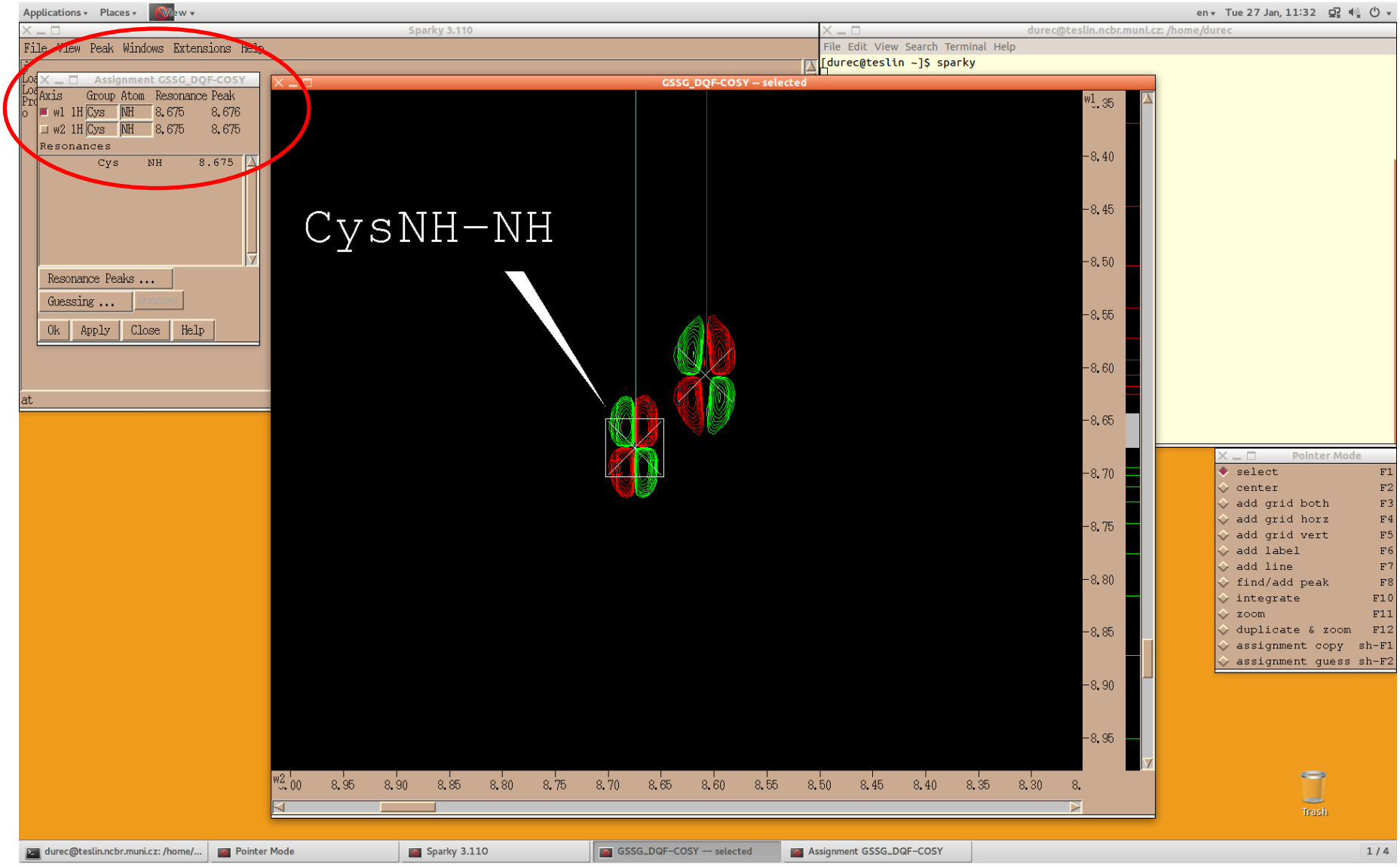
Gly – 2: Hα, NH

Cys – 4: Hα, NH, Hβ‘, Hβ‘‘

Glu – 3: Hα, Hβ, Hγ

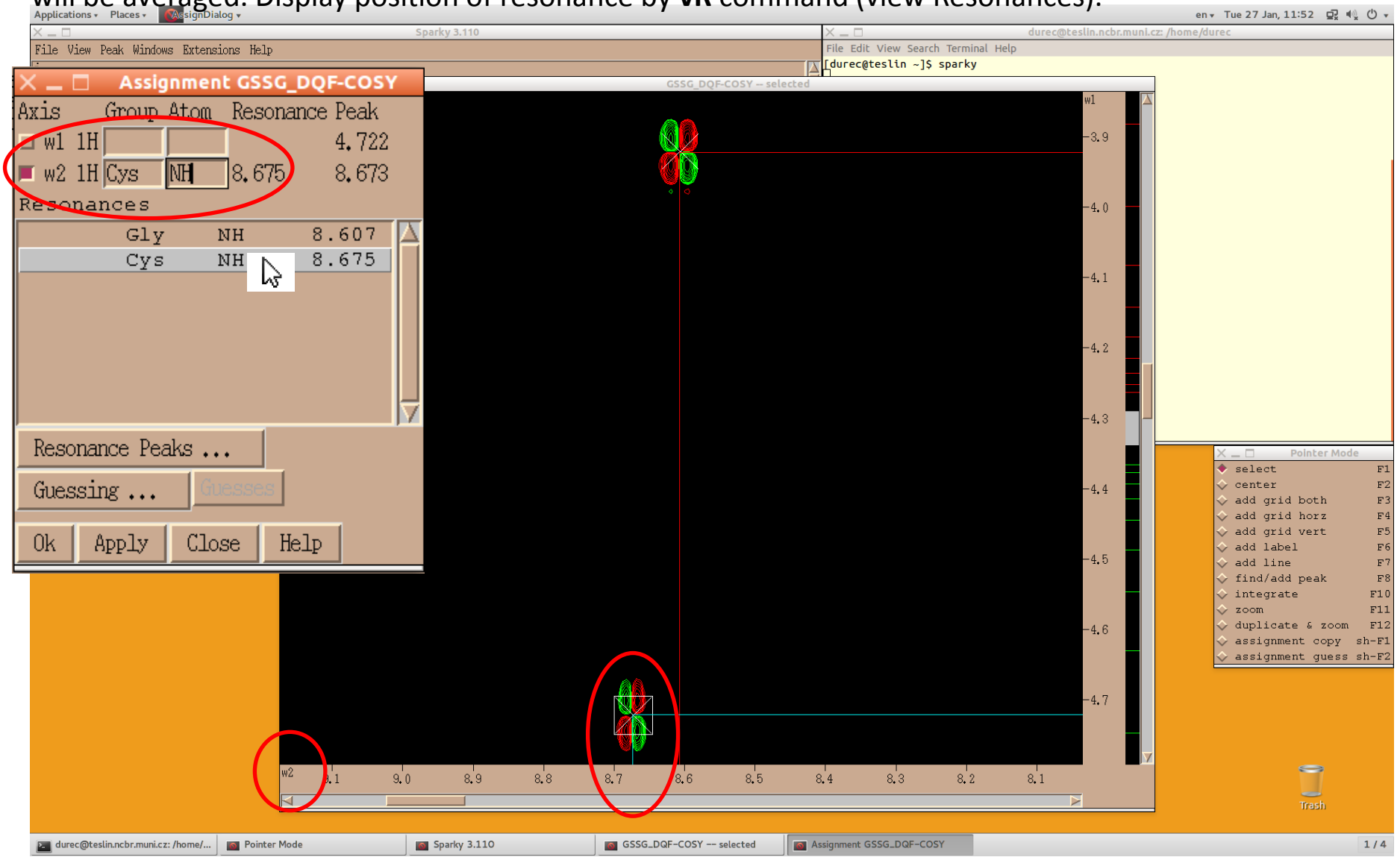
Signal assignment:

select the peak: pointer mode – select: key F1 → shortcut ***at*** – assignment → fill in the name of the group (e.g. abbreviation of residuum/molecule) and the name of the atom (eg. NH) according to the resonance in w1 and w2 axis → type apply



Signal assignment:

the assignment with the closest resonance to the current selected resonance is highlighted when clicking on the name of the axis. (not necessarily correct one!!), double click on the highlighted line adds the name of the **group** and **atom label** to currently selected peak. Use unique naming, otherwise resonances will be averaged. Display position of resonance by **vR** command (view Resonances).

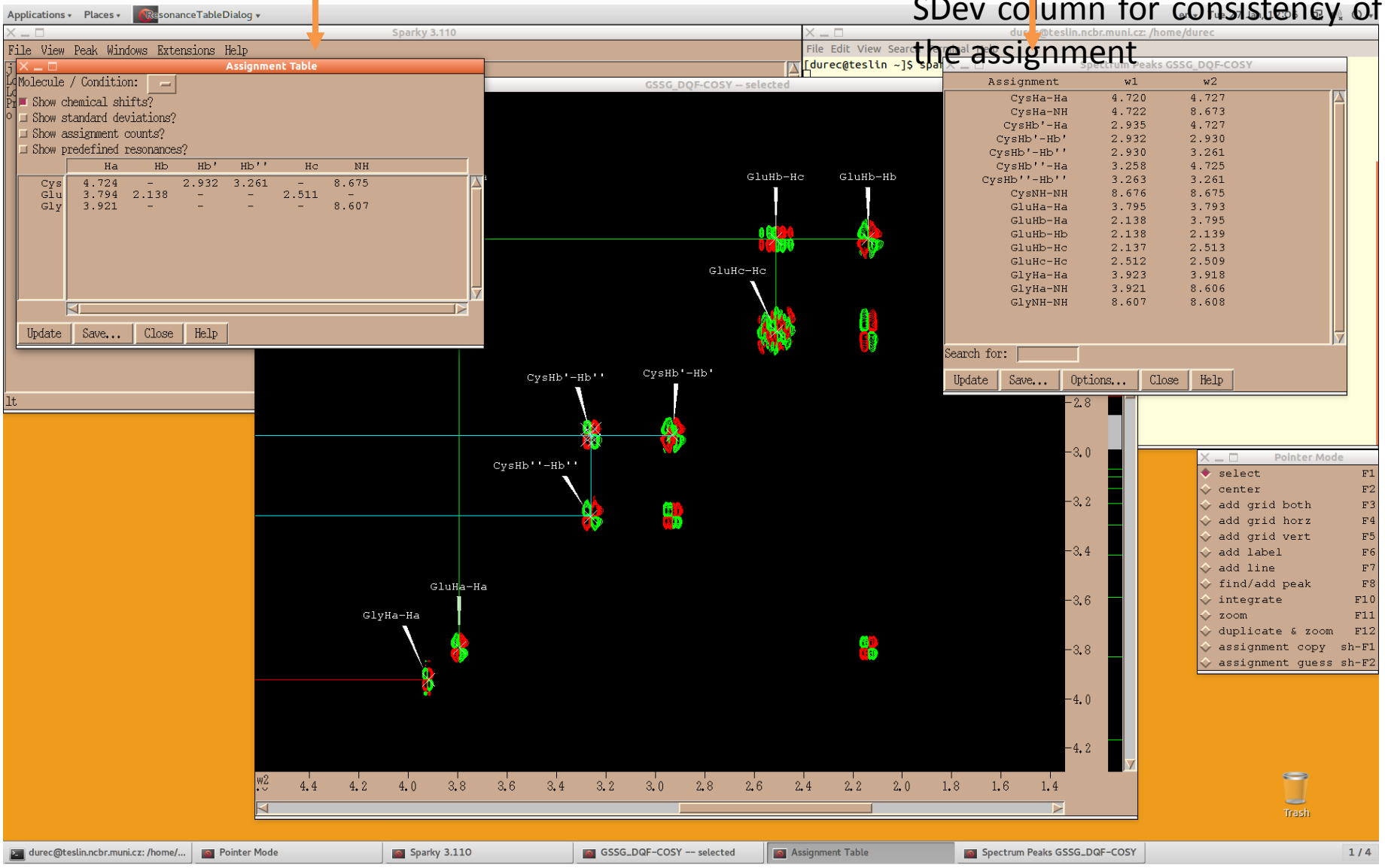


signal assignment:

shortcut ***tb*** – assignment table residues vs. atoms, statistical data can be shown as well

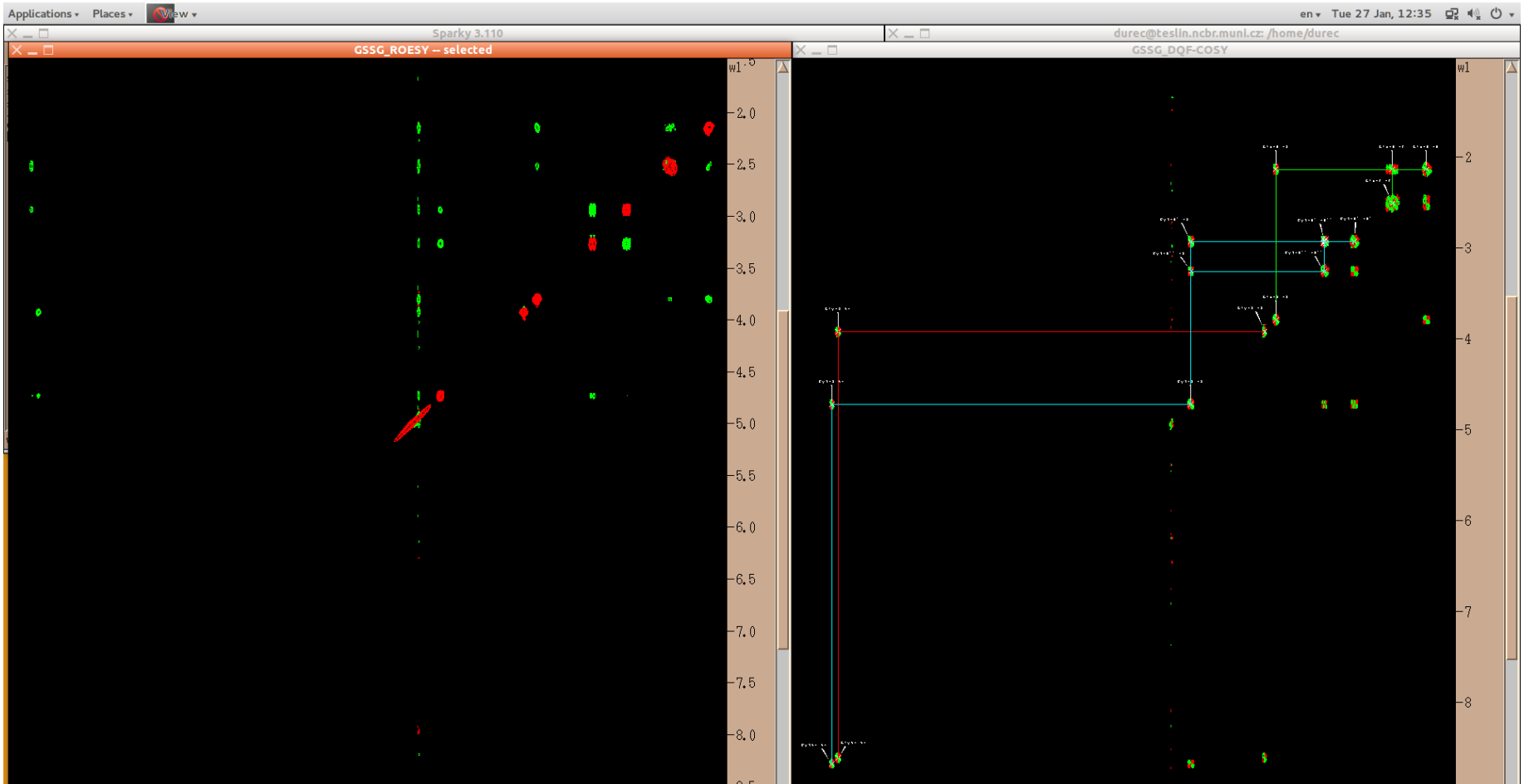
both can be exported as a plain text file (→ Save)

shortcut ***lt*** – list of the peaks already with assignments, or with other information (→ Options) ! Always check SDev column for consistency of the assignment



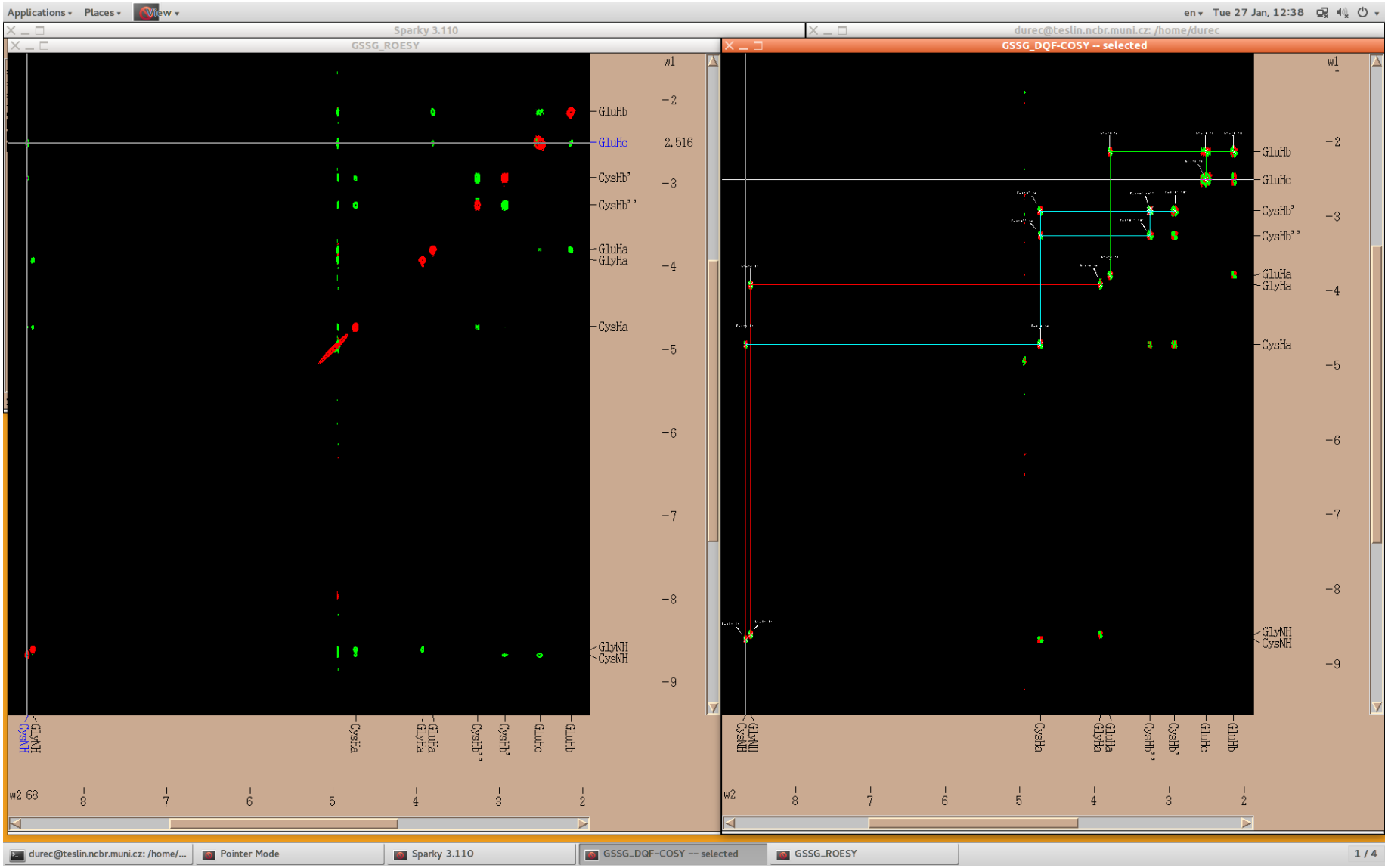
Working with more spectra:

Assignment made in on spectrum is available/shared also for other spectra, peak assignment can be completed using different experiments on condition that experiments were recorded at the same temperature, identical sample etc. During work you may save separately the spectra (file save – experiments of active window) and subsequently save the entire project



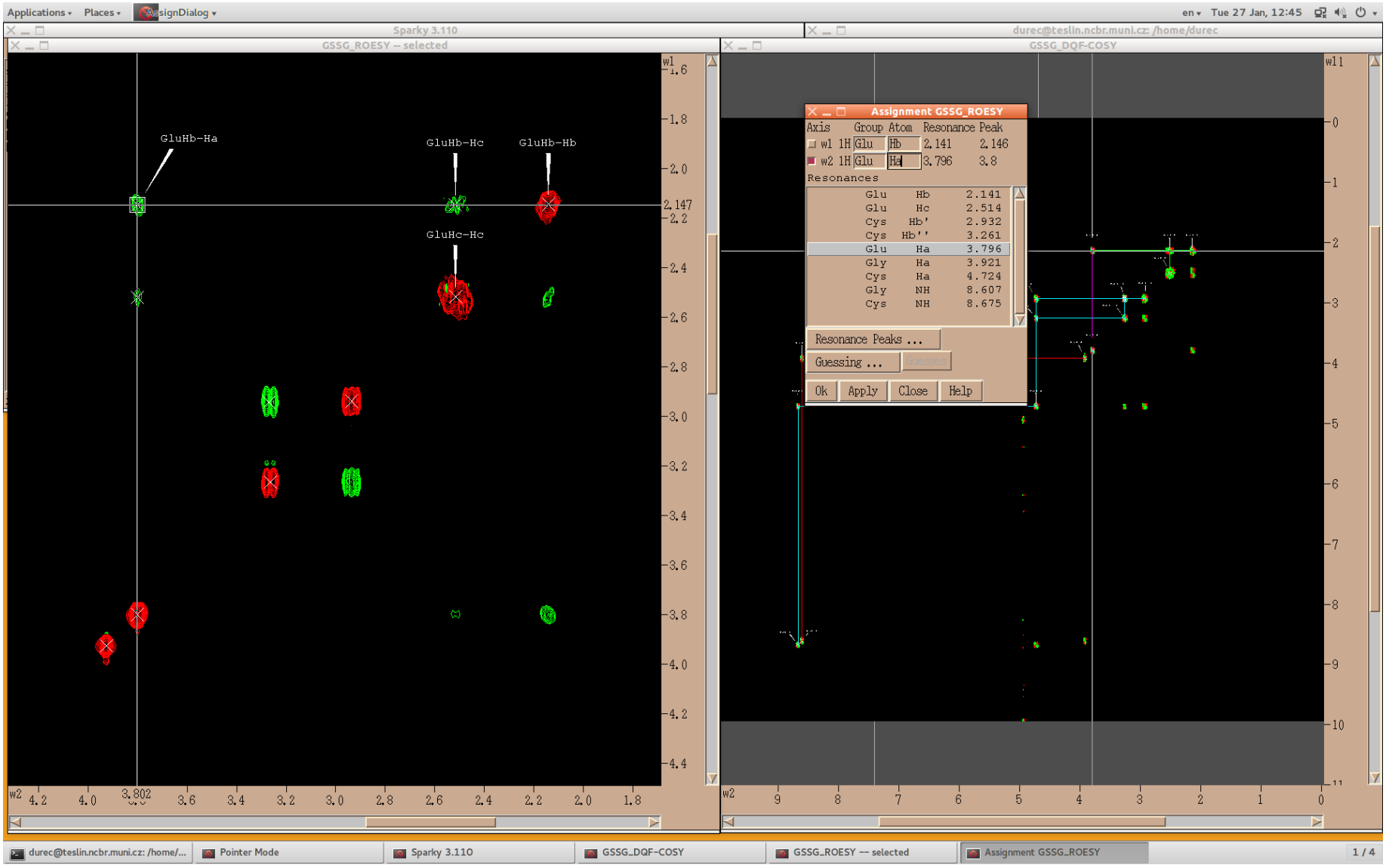
working with more spectra:

view assigned (averaged) resonances on the edge of the spectra - ***vR***



working with more spectra:

assign ROESY spectrum by using ***at:***



working with more spectra:

synchronize views of spectra: shortcut ***yt*** – it allows simultaniously move synchronized axes of spectra when moving one of them,

click on the axes in spectra we want synchronize → Synchronize