ThreeAngleOTF

From Micron

Contents

Data acquisition

Locate a bead with a clean background at least 1/4 of the field clear around it (even away from focus).

Laser: 488nm

Camera: wheel

Filter: GFP (515)

- Set the laser power to 40mW and exposure time to 5ms.

- Set the camera as follows:
  - EM 5MHz
  - EM gain 200
  - clean, no FT (this is the default setting)

- Click Single-site experiment
- Set up as follows:

- Filename
  - suffix should be 'PSF-[excitation]-[emission]'
    - **THERE MUST BE NO SPACES IN THE FILENAME ...** some bits of softWoRx hate spaces.
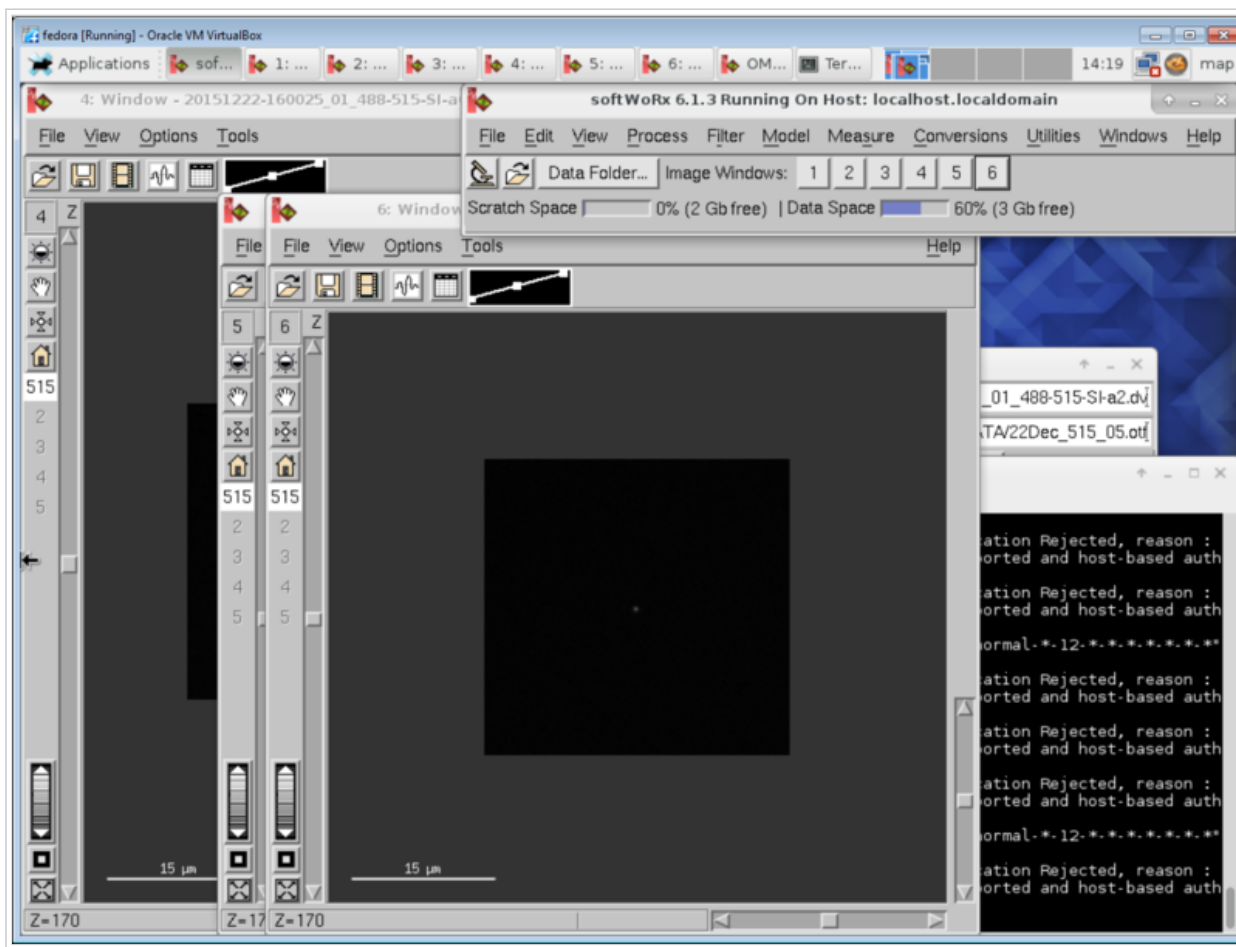- Click 'Update' to set timestamp on file.
- Click 'Start' to run.

Analysis

- Open the VM.
- **NEVER CLOSE softWoRx**
- **NEVER LOG OUT OF THE VM**
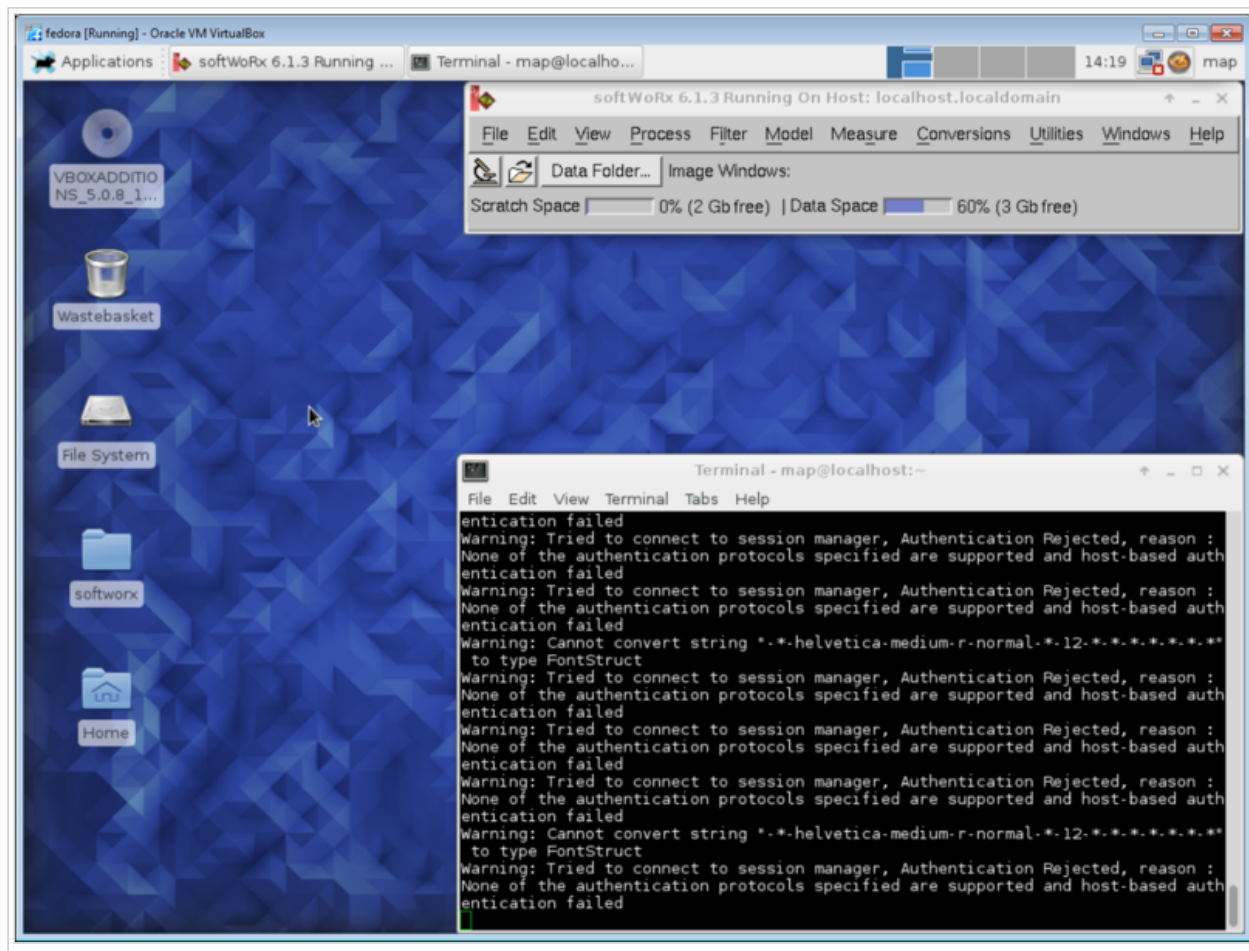- **TO CLOSE THE VM, CLICK THE TOP RH 'X', CHOOSE 'SAVE MACHINE STATE' THEN CLICK 'OK'**
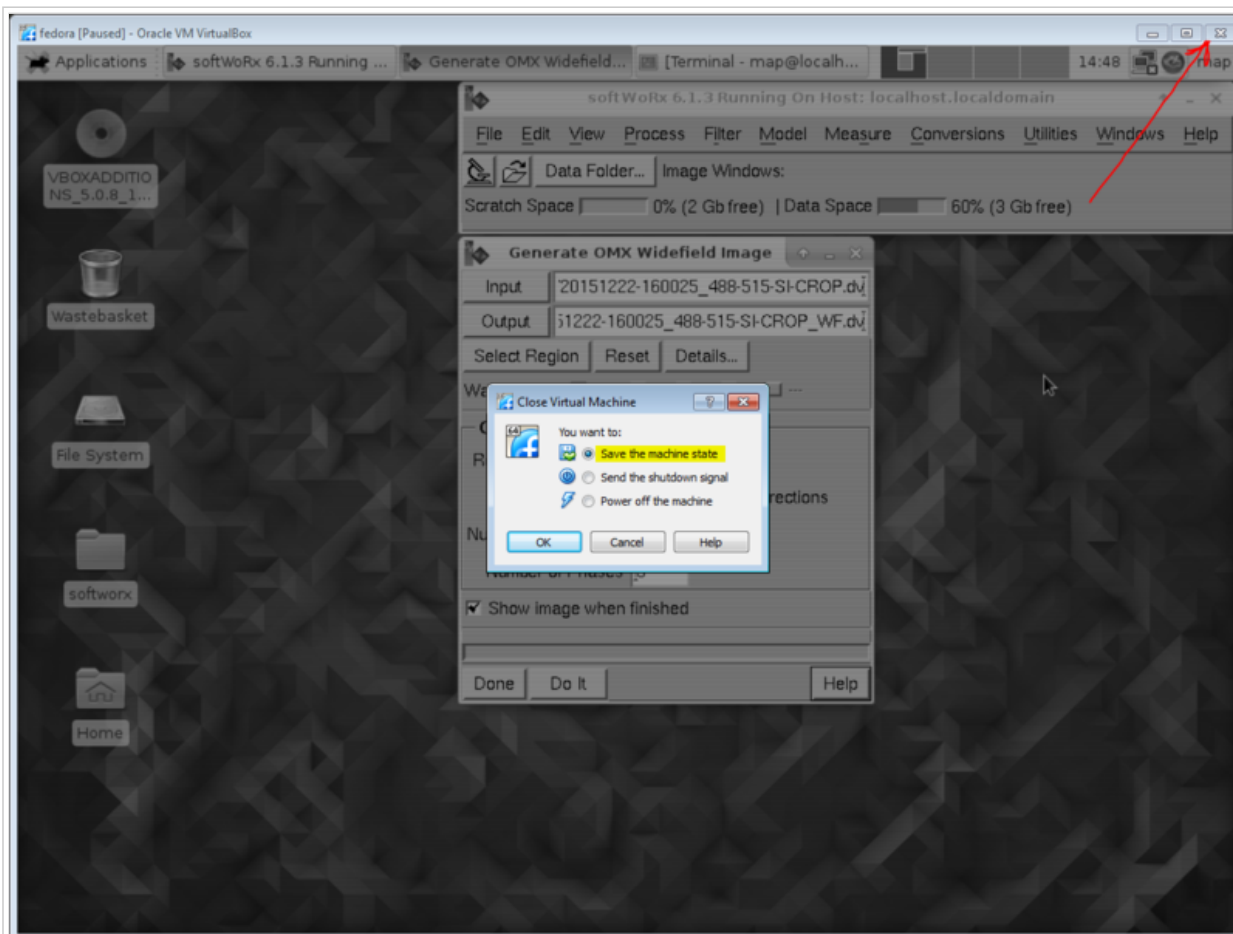
Closing the VM

- username: map
- password: micron1
- softWoRx will be running.

- Close any open image windows. **DO NOT CLOSE THE MAIN SOFTWORX WINDOW OR TERMINAL**

- In the softWoRx window:
  - File->Open
  - Open the data file you acquired.
- This opens an image window.
- Use the image window left scrollbar to shift focal plane and find your bead.
- Image window: File->Save
- Drag the save window to the right of the image window.
- Save window: Select Region
- Drag a red box around the bead in the image window.
- Save window: Details...
- Region details window: adjust X/Y/Width/Height to centre bead in a 256x256 box
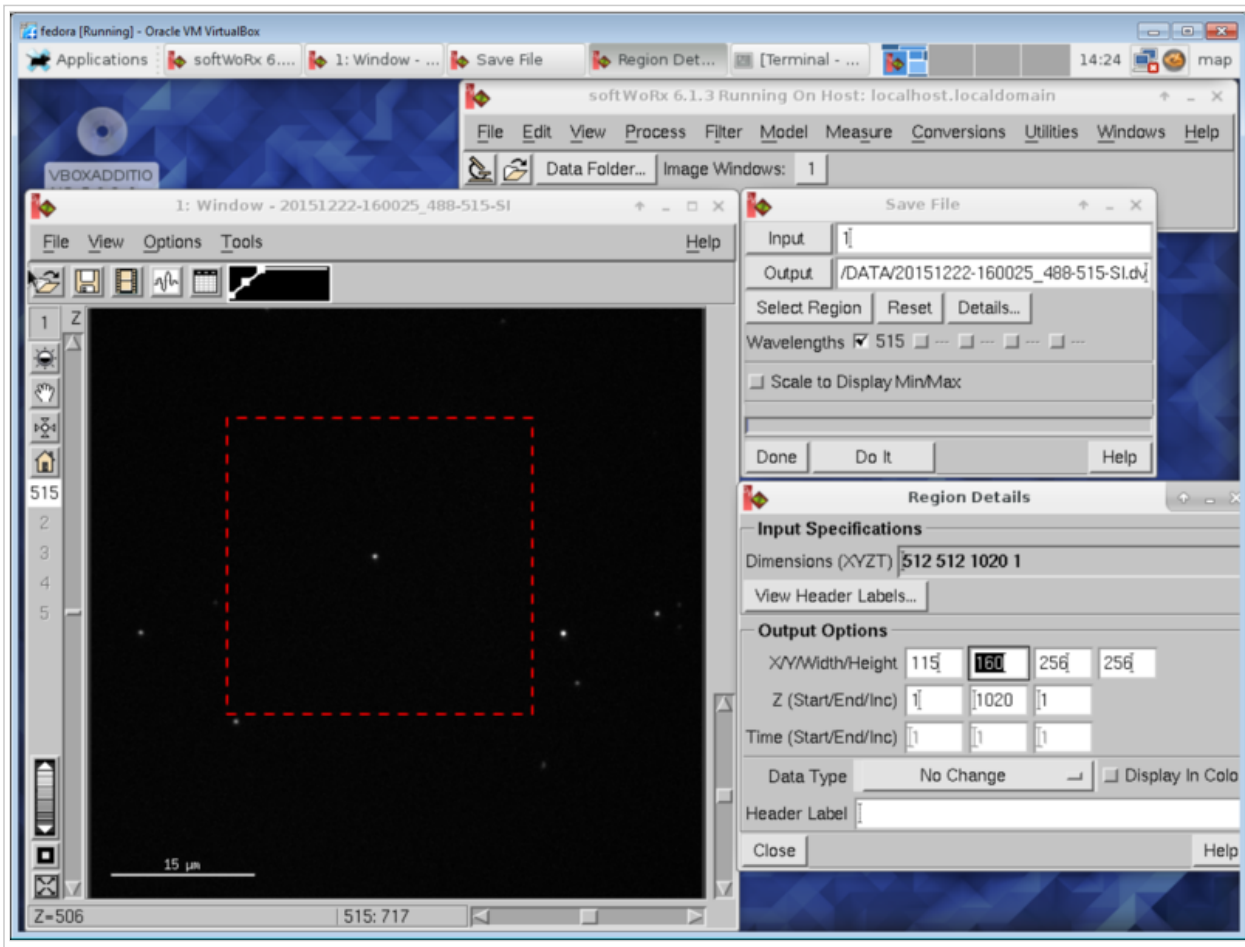
- Region details window: close
- Save file window: add '-CROP' in 'Output' between 'SI' and '.dv'
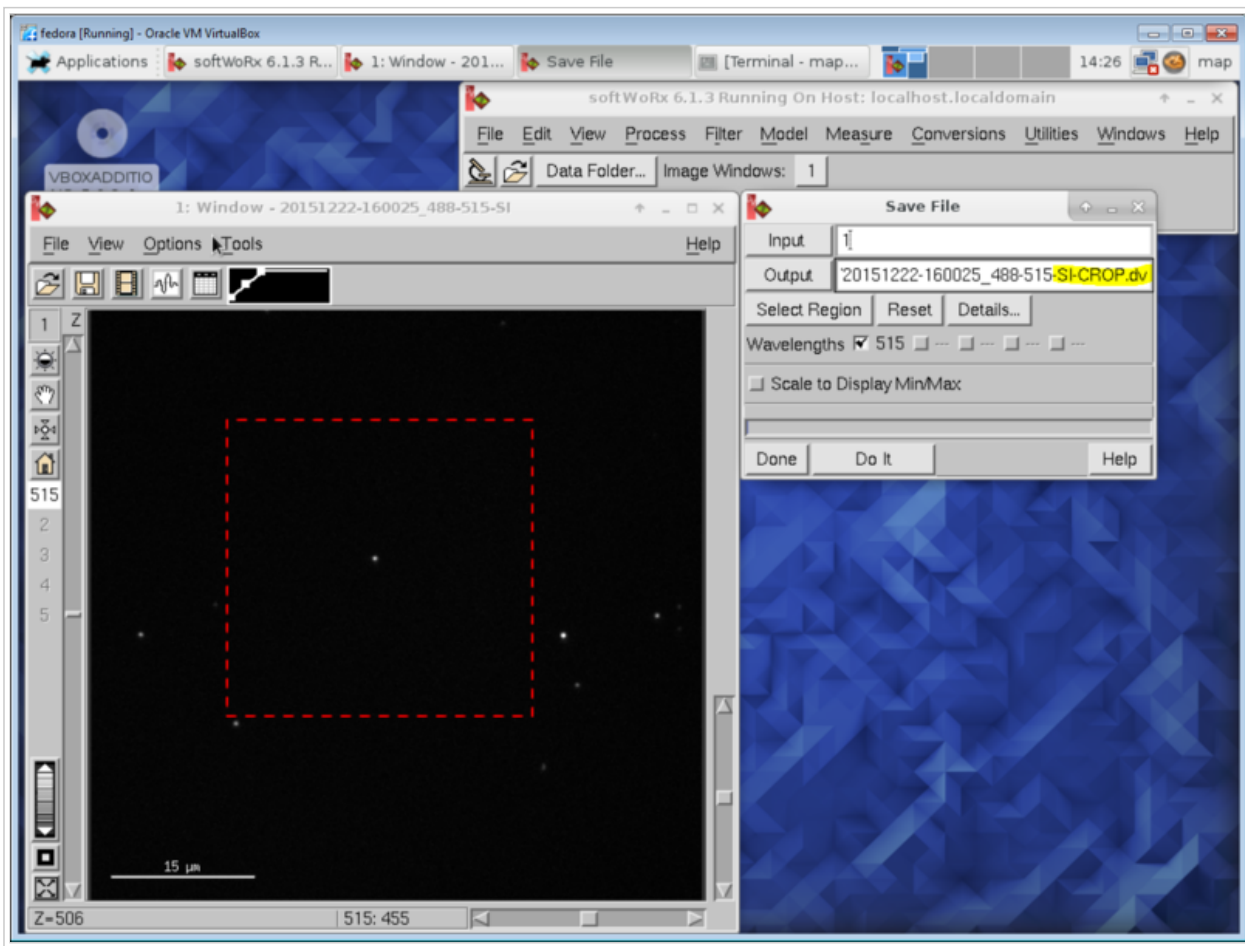
- Save file window: 'Do it'
- This:

- saves the cropped region to a new file,
- closes your original file,
- opens the crop file.
- Close the crop file for now.

Widefield PSF generation

- softWoRx window: Process->Generate Widefield from SI Image
- This opens the 'Generate OMX Widefield Image' window.
- Click 'Input' and choose the crop file.
- This also populates the Output filename field - leave it as it is.
- Check the 'Average directions' box.
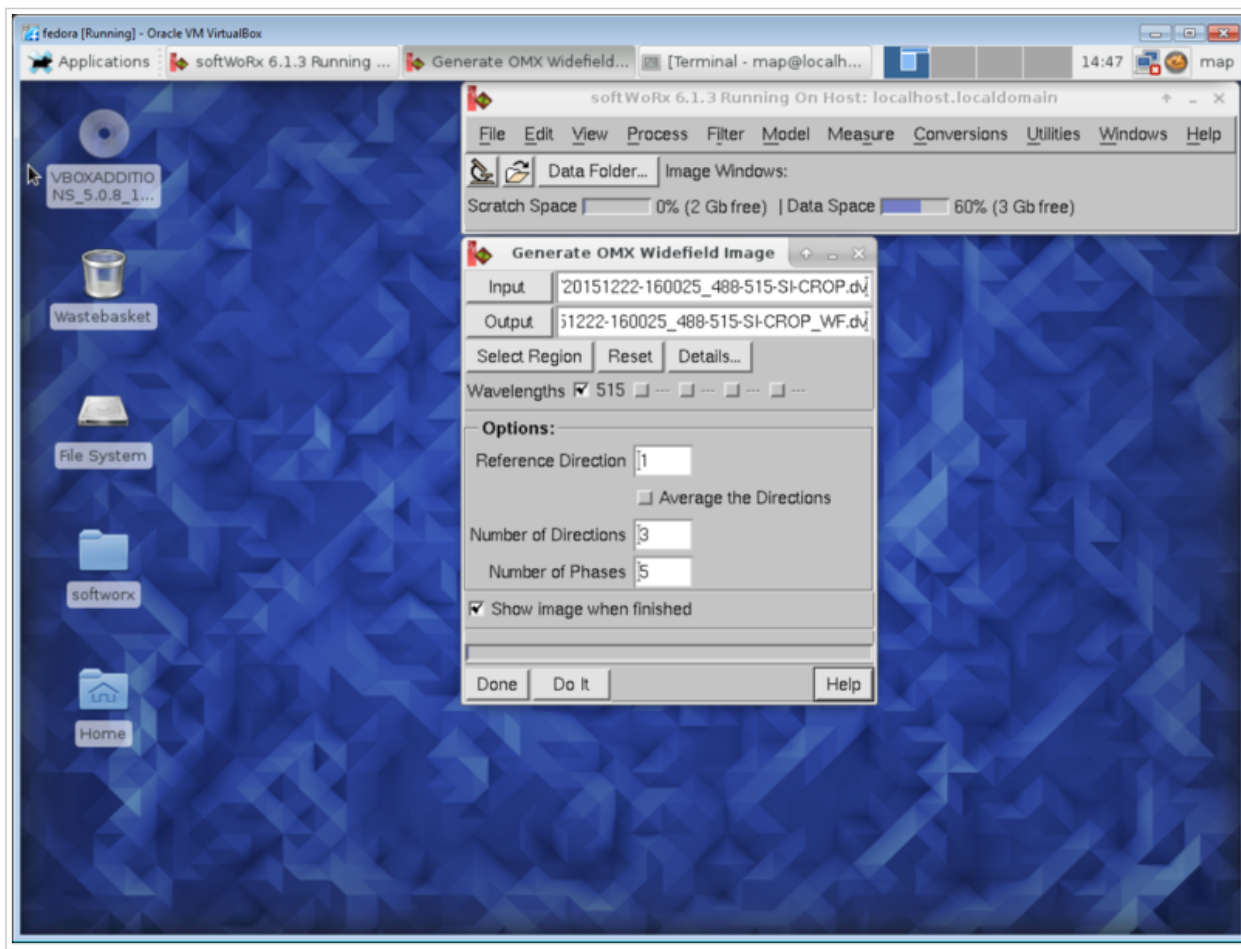
- Click 'Do it'.
- This closes your cropped image and opens the pseudo-widefield (WF) image.
- Close the pseudo-WF window.

Angle extraction

- This will repeat 3 times - once for each angle.
- Open the cropped image.
- Cropped image window: File->Save
- Save window: Details...
- Details window
  - Find 'Z row: Start/End/Inc'
  - make a note of End (should be 1140 if following acquisition instructions)
  - First run through:
    - set End to original value / 3 (380)
  - Second run through:
    - set Start to (original value / 3) + 1 (381)
    - set End to (2 * original value / 3) (760)
  - Third run through:
    - set Start to (2 * original value / 3) + 1 (761)
    - set End to original End value (1140)
- click 'Close'
- Save window:
  - First run through:
    - replace 'CROP' in filename with 'A0'
  - Second run through:
    - replace 'CROP' in filename with 'A1'

- Third run through:
    - replace 'CROP' in filename with 'A2'
- click 'Do it'
- This:
    - saves angle-N slices to new file
    - closes the crop file
    - opens the a{0,1,2} file
- Reopen the crop file and repeat from **Angle extraction** for a total of 3 times.

OTF generation

- For each angle file:
    - softWoRx window: Process -> Make OMX OTF
        - Opens the OMX Create OTF window.
    - OMX Create OTF window:
        - click PSF-File, choose an angle file
        - This creates a filename in the OTF file box.
        - Change the OTF file name so that it ends with the angle number (A0, A1, A2)
        - Uncheck 'Leave KZ'
        - Uncheck 'Do bead compensation calculation'
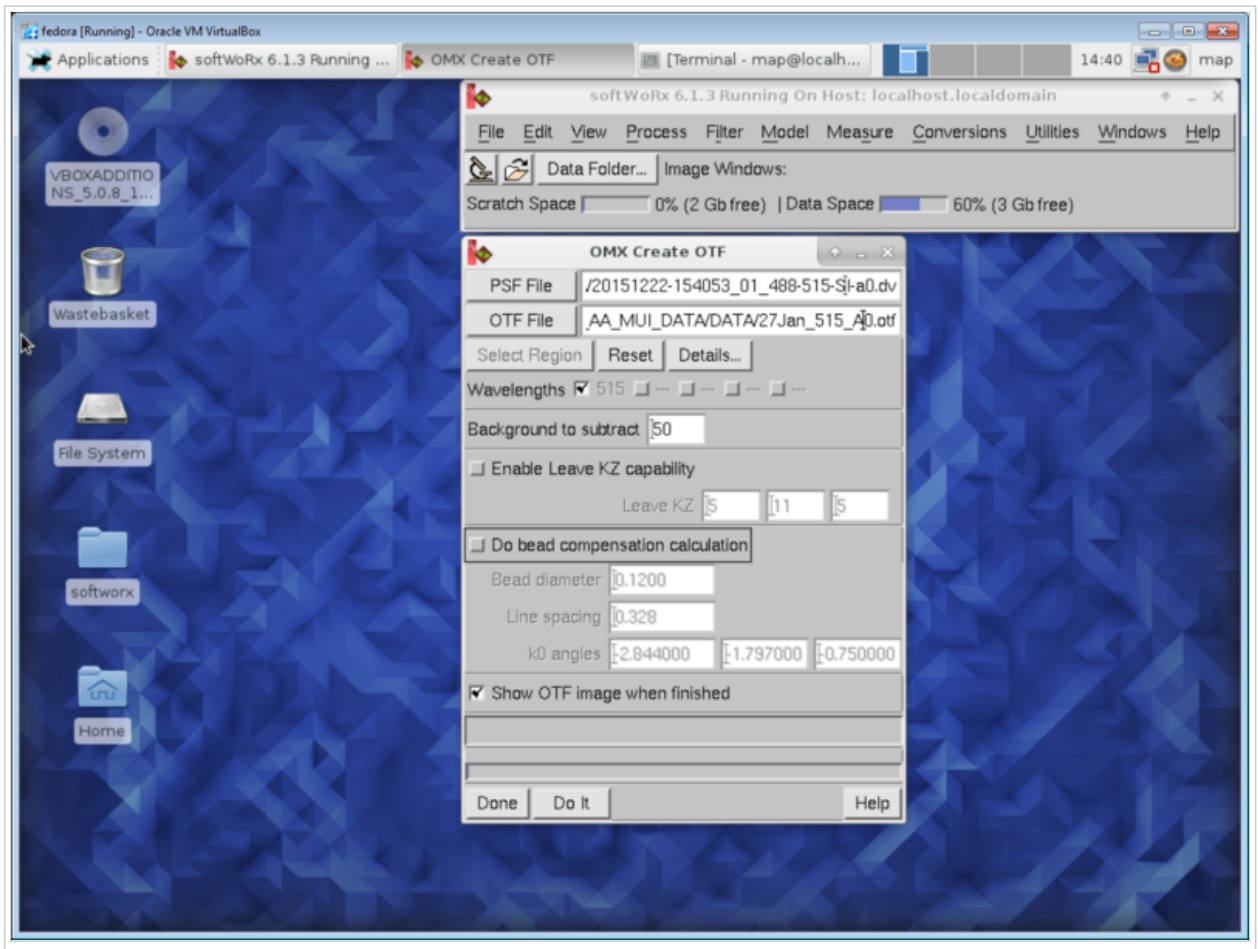
- Click 'Do it'

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