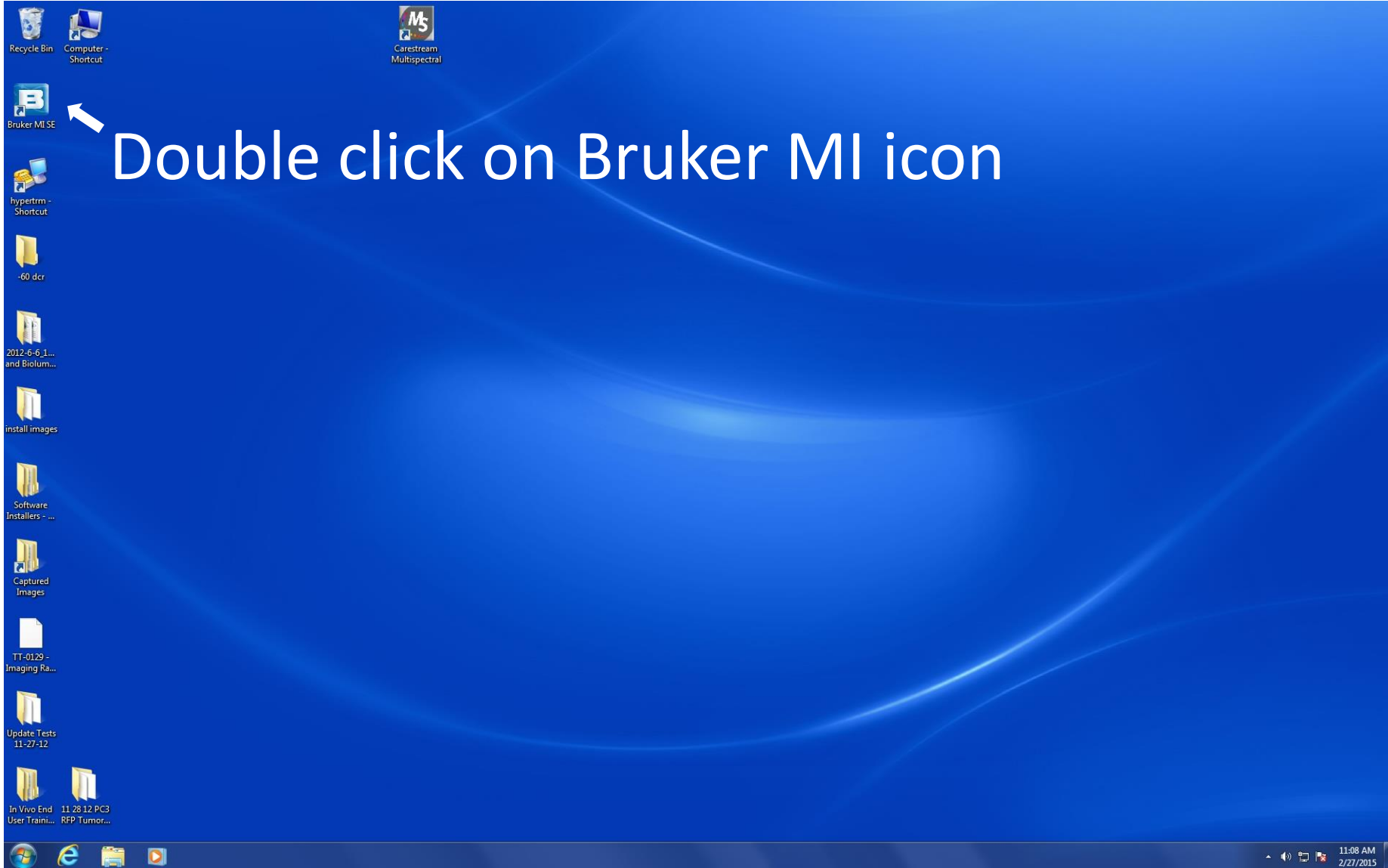
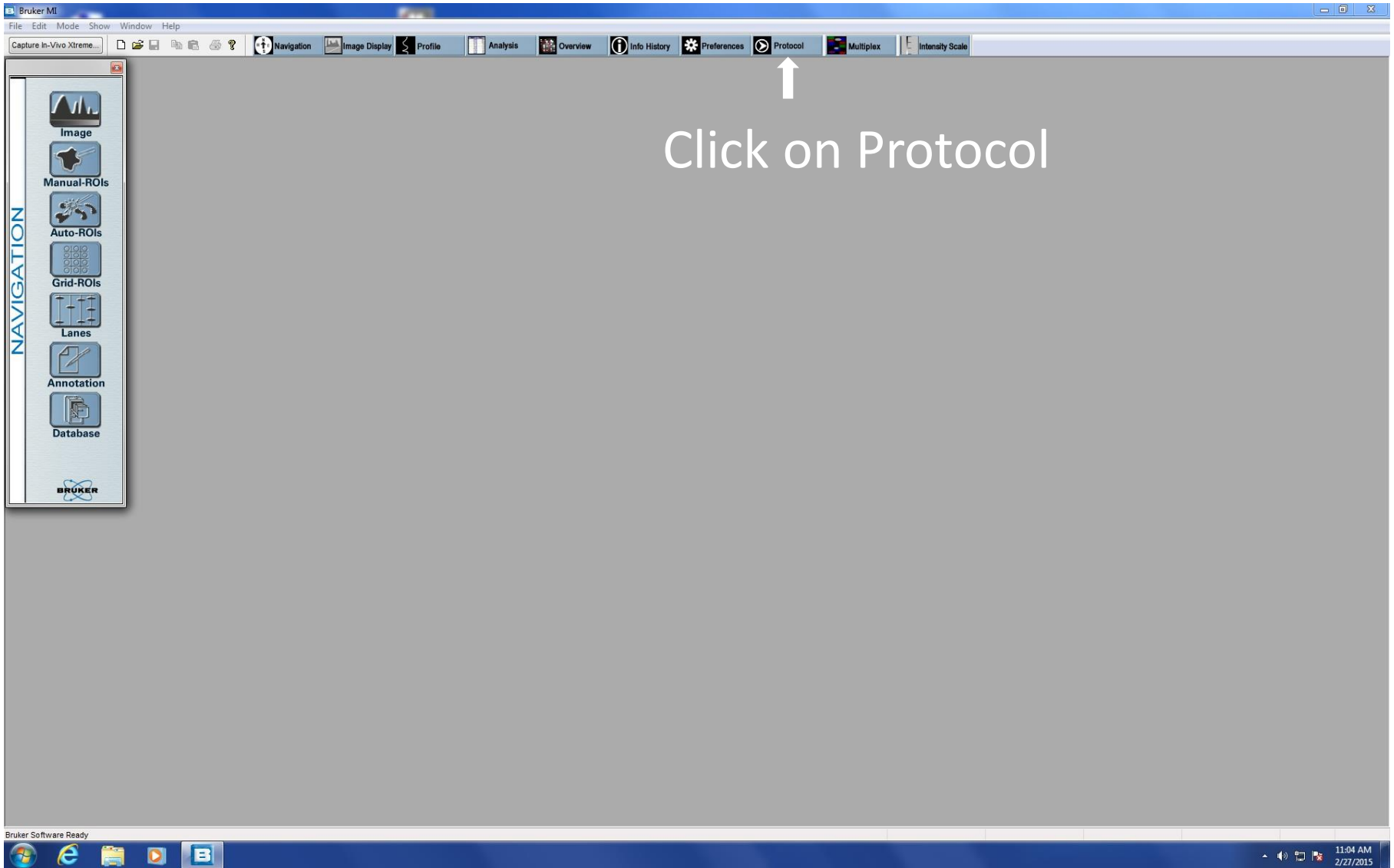


Tutorial for running Bruker
Molecular Imaging Camera to
measure red fluorescent protein
fluorescence in mice



Double click on Bruker MI icon

Bruker MI program opens up and will look like this. Click on Protocol.



To measure red fluorescent protein (RFP) for 5 mice, use the Tumor-RFP protocol. If this protocol does not come up automatically when you click on Protocol (described in previous slide), click the arrow in the drop down box for Protocol name.

The screenshot displays the Bruker MI software interface. On the left is a vertical 'NAVIGATION' sidebar with icons for Image, Manual-ROIs, Auto-ROIs, Grid-ROIs, Lanes, Annotation, and Database. The main window shows the 'Protocol' configuration for 'Tumor-RFP'. The 'Protocol Name' dropdown is highlighted with a white arrow and the text 'Protocol name →'. A callout box points to this dropdown with the text: 'Click on arrow for drop-down box, find the protocol Tumor-RFP, click on it to get to Tumor-RFP.' Another callout box points to the 'Browse' button next to the 'Save Images To' field with the text: 'Click "Browse" to direct the program to the path where the camera image files for this particular protocol will be saved in a folder.' The 'Protocol Steps' section shows 'Step 1' selected, with options for 'Capture New Images' and 'Use Existing Images'. The 'Capture Setting' is set to 'MWL_20.000Sec_0.00cm_Ex530Em600_BinX2Y2'.

Protocol name →

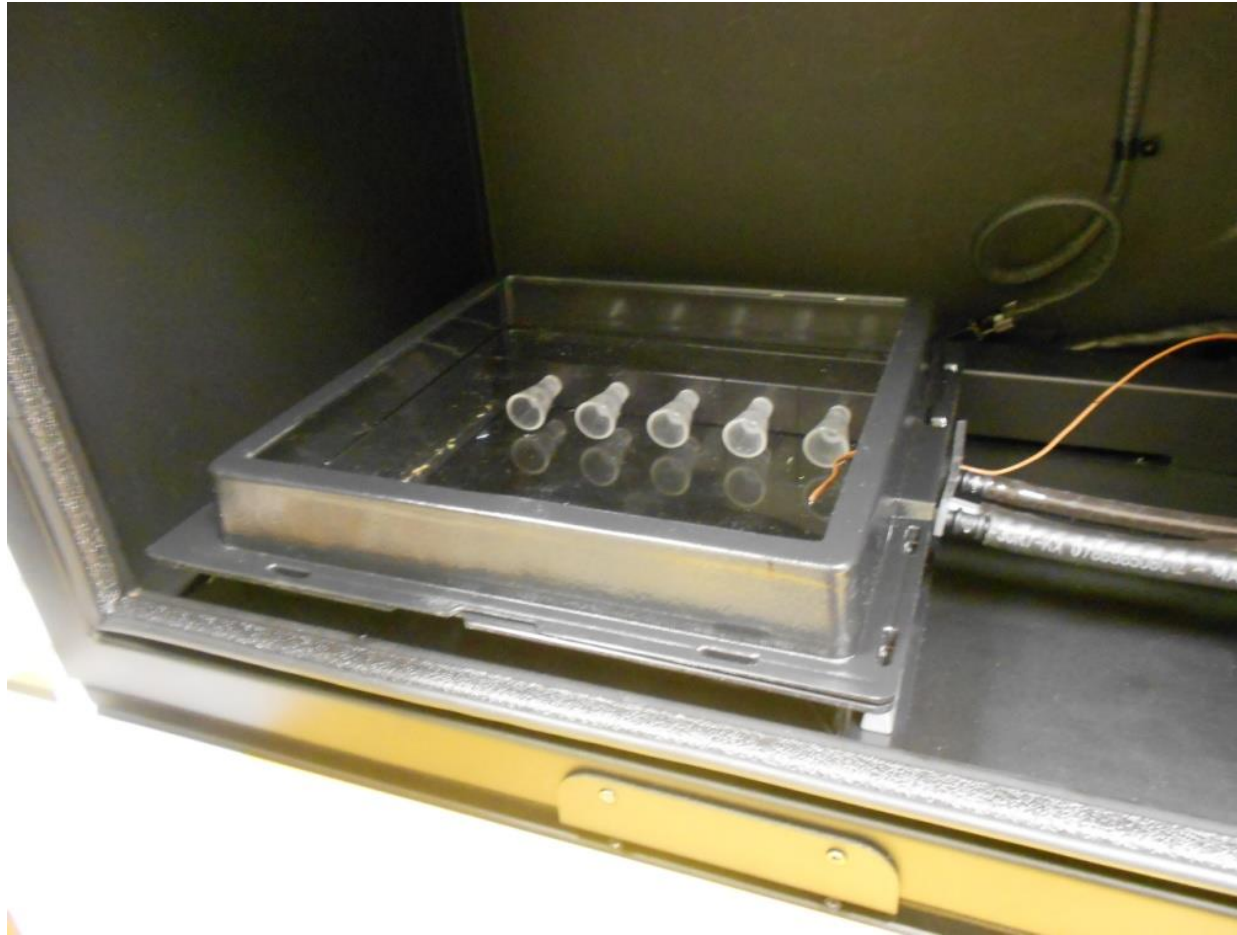
Click on arrow for drop-down box, find the protocol Tumor-RFP, click on it to get to Tumor-RFP.

Click "Browse" to direct the program to the path where the camera image files for this particular protocol will be saved in a folder.

We have found that the easiest way to get mice asleep and to put their snouts in nozzles in the camera image cage is use the following steps (also described in slides 6-11):

- 1) Make sure image cage in camera box is closed and sealed.
- 2) Make sure enough anesthetizing drug is in anesthesiologist machine.
- 3) Turn on oxygen to anesthesiologist machine.
- 4) Unlock drug control by pushing down black button on control and set drug delivery control to 5.
- 5) Wait a couple of minutes for camera image cage to fill with drug.
- 6) Add mice and close cage so that it is sealed as soon as mice have been placed in cage. We have found that it is easiest to add 3 mice, let them fall asleep then add the other two mice.
- 7) Once all mice have fallen asleep, open cage and position mice in desired order with their snouts in nozzles. Tumors should be closest to the floor. The camera is below the floor. Try to do this quickly so mice do not wake up, but you may have to close up and seal cage and let mice fall asleep again before you can finish this step.
- 8) Once mice are asleep with snouts in nozzles and tumors closest to the floor of the cage, close door of camera cage. Make sure door to camera does not say "Door open" when you close door. If the door message does say "door open", open door and close it again until the message does not appear.
- 9) Turn drug control down to 3.5.
- 10) Click the execute button in the protocol.

- 1) Make sure image cage in camera box has drug delivery connected and cage is closed and sealed.



← Drug delivered through this tubing. Make sure it is connected to nozzle manifold in cage.

- 1) Make sure image cage in camera box has drug delivery connected and cage is closed and sealed.
- 2) Make sure enough anestheisizing drug is in anesthesiologist machine.
- 3) Turn on oxygen to anesthesiologist machine.
- 4) Unlock drug control by pushing down black button on control and set drug delivery control to 5.
- 5) Wait a couple of minutes for camera image cage to fill with drug.

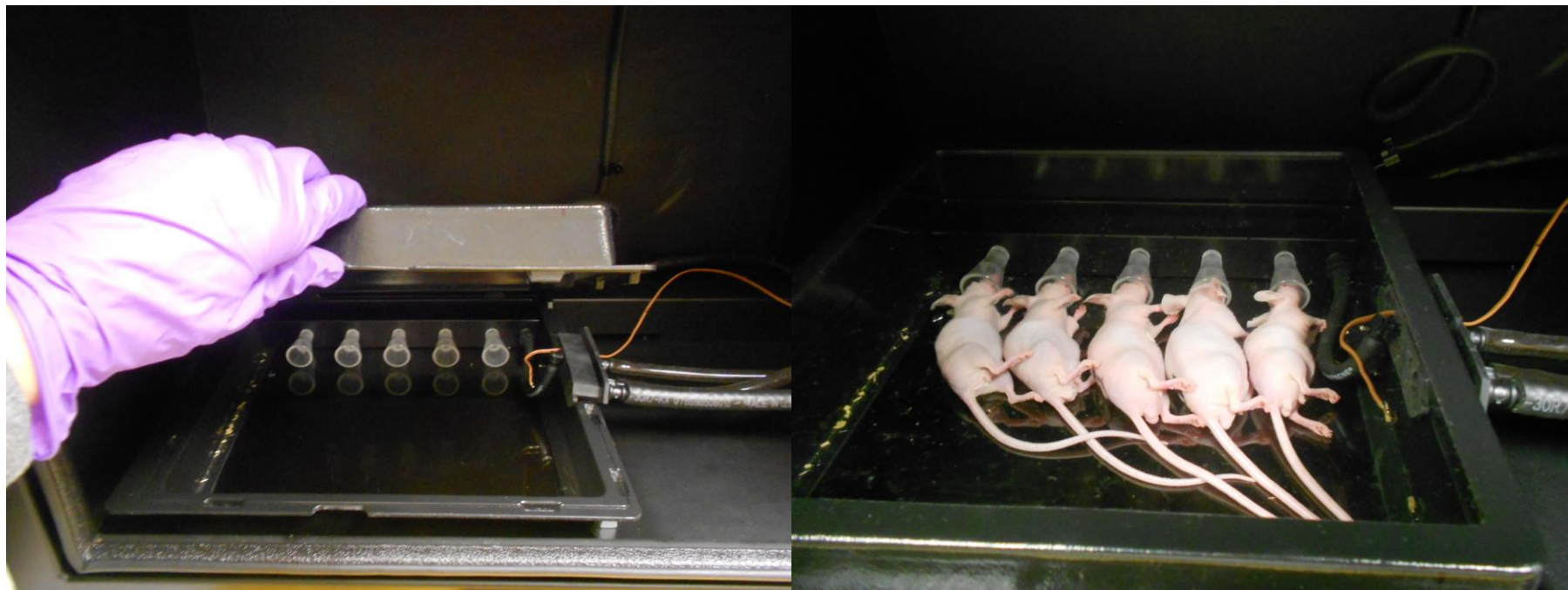
Anestheisizing machine



Turn on oxygen by turning black handle clockwise. Turn off by turning handle counter-clockwise.



- 6) Add mice and quickly close cage so that it is sealed as soon as mice have been placed in cage. We have found that it is easiest to add 3 mice, let them fall asleep then add the other two mice.
- 7) Once all mice have fallen asleep, open cage and position mice in desired order with their snouts in nozzles. Tumors should be closest to the floor. The camera is below the floor. Try to do this quickly so mice do not wake up, but you may have to close up and seal cage and let mice fall asleep again before you can finish this step.

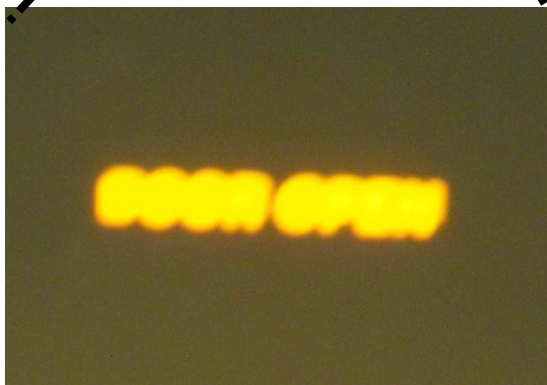


Quickly open cage and put in mice. Let mice fall asleep before trying to position them.

- 8) Once mice are asleep with snouts in nozzles and tumors closest to the floor of the cage, close door of camera cage. Make sure door to camera does not say “Door open” when you close door.

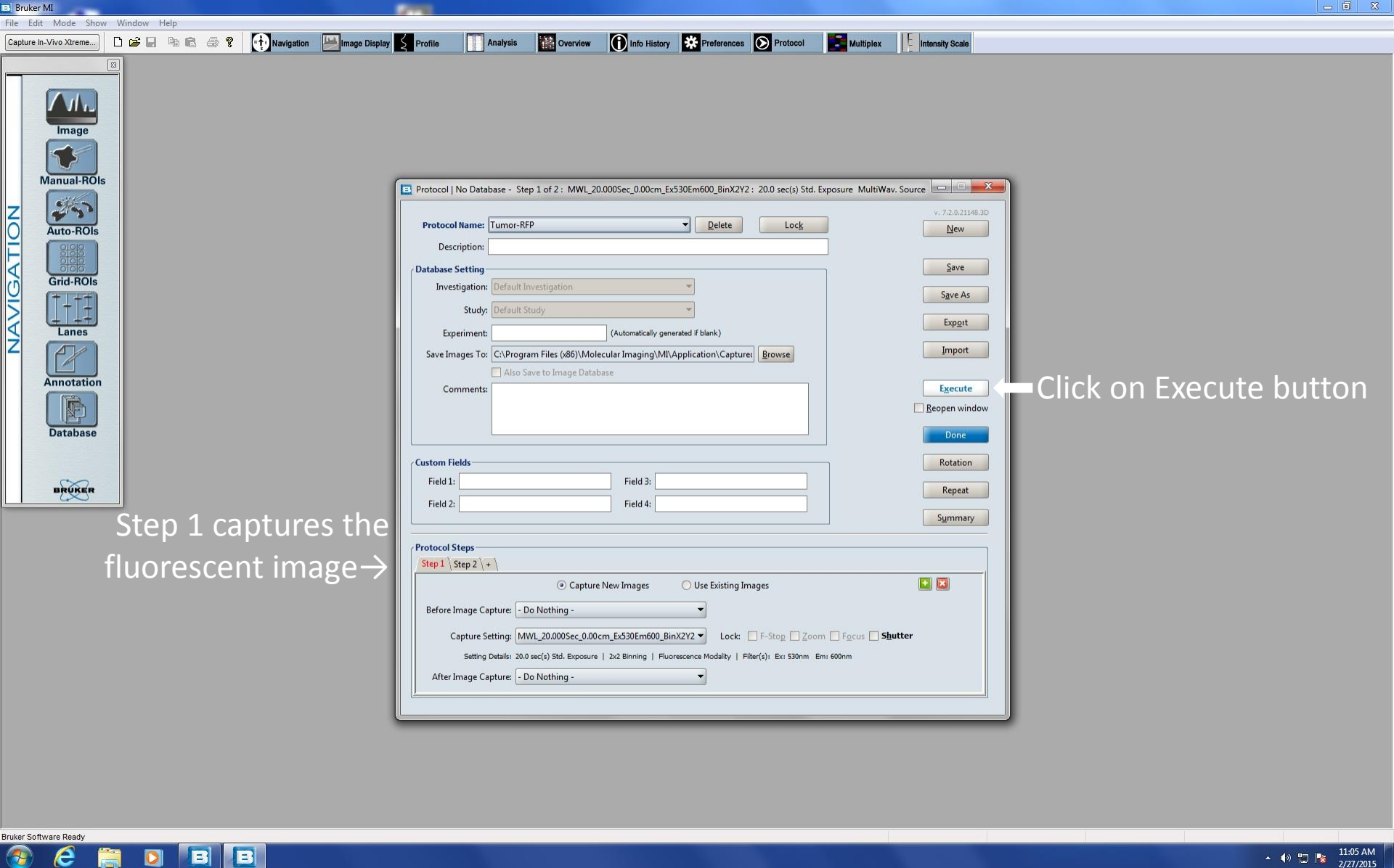


If the door message does say “door open”, open door and close it again until the message does not appear.



9) Turn drug control down to 3.5.

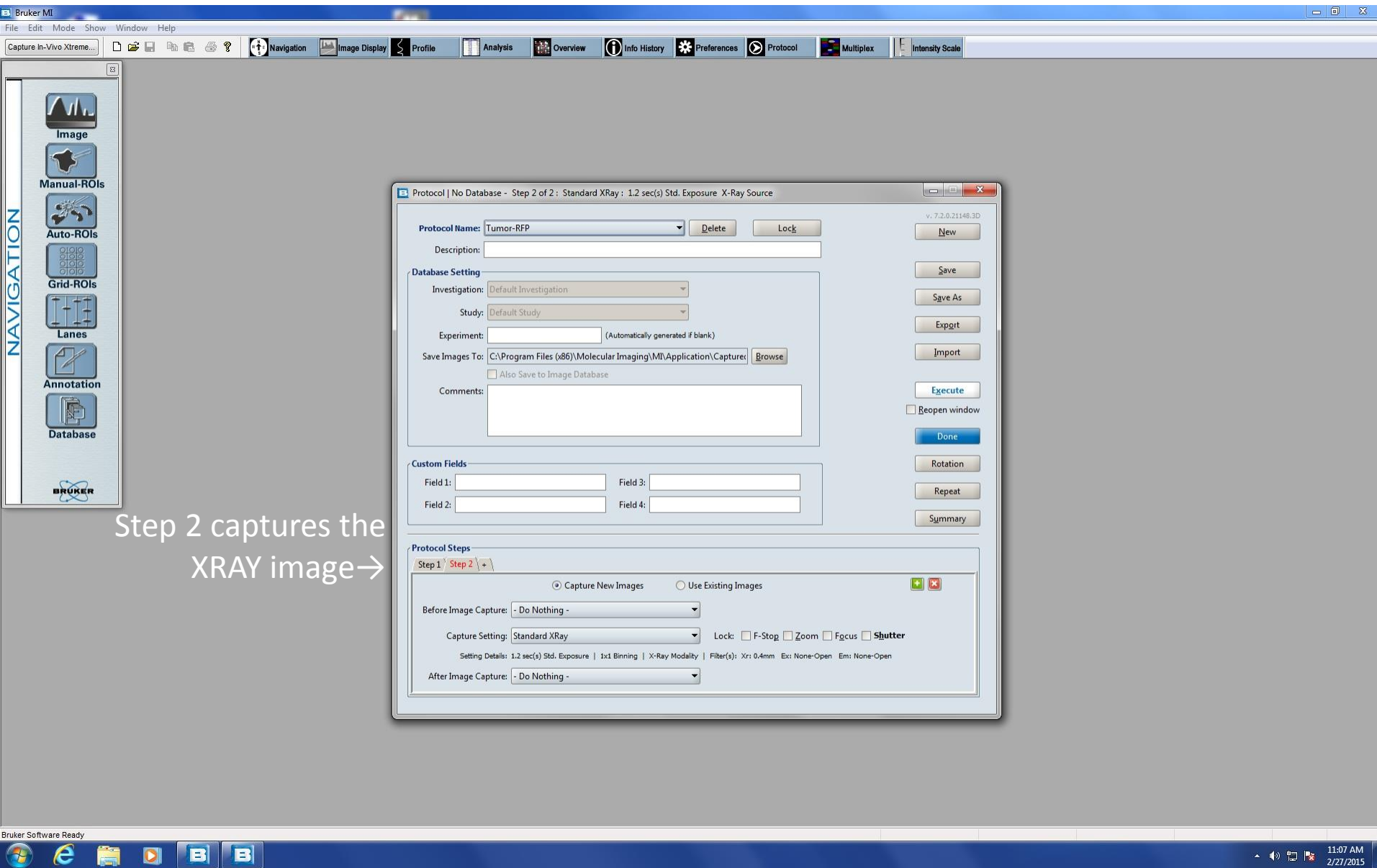
10) Click the execute button.



Step 1 captures the fluorescent image →

Click on Execute button

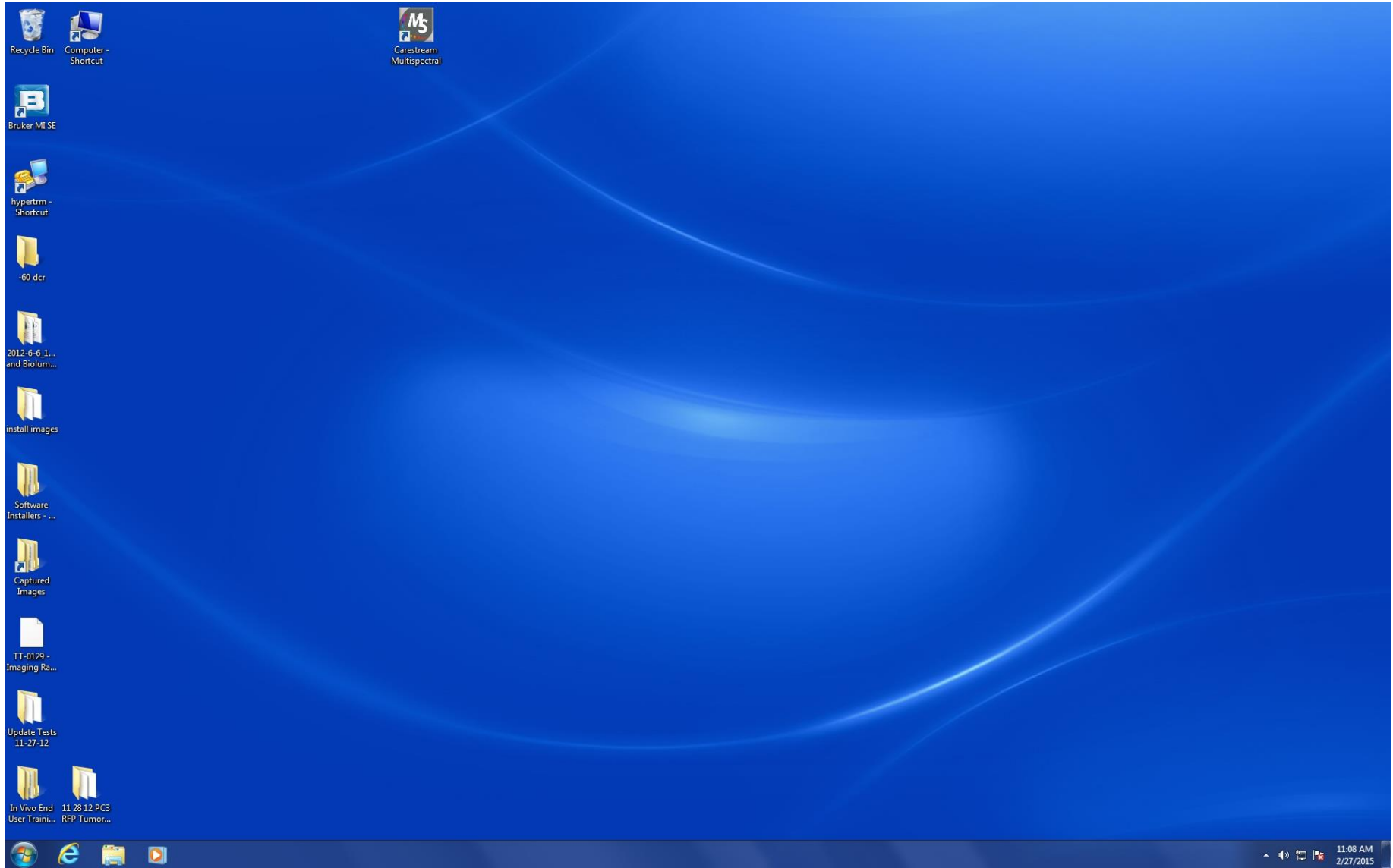
Step 2, shown below, is automatically performed after step 1. Step 2 captures the XRAY image. Make sure the program has completed downloading both images before opening the door of the camera.



Step 2 captures the XRAY image →

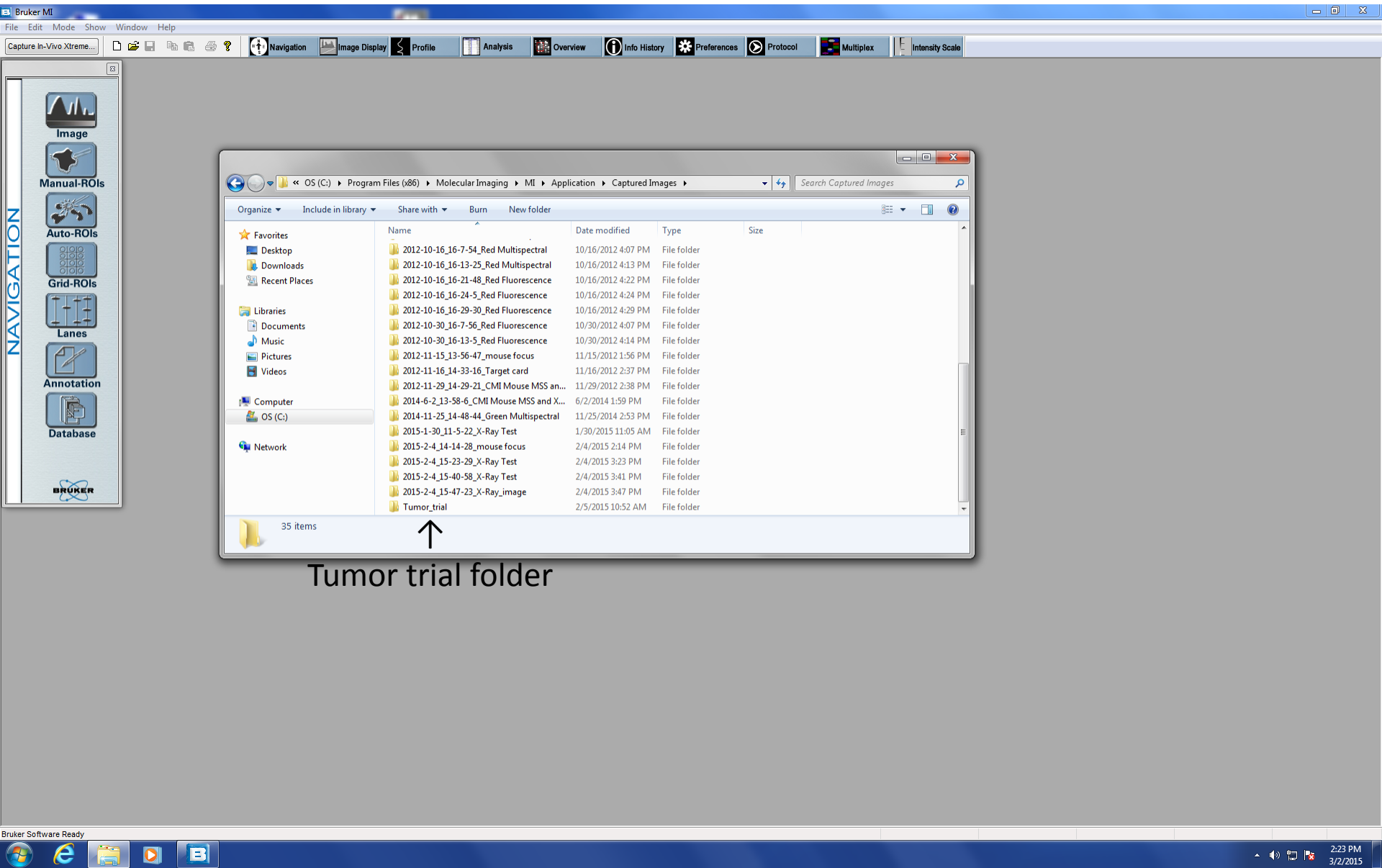
When you are through taking images and mice have been removed and placed back in their housing cages, don't forget to turn off the oxygen and set the drug delivery to zero.

To look at images using the Bruker program, click on the Windows Explorer folder icon at the bottom of the computer screen.

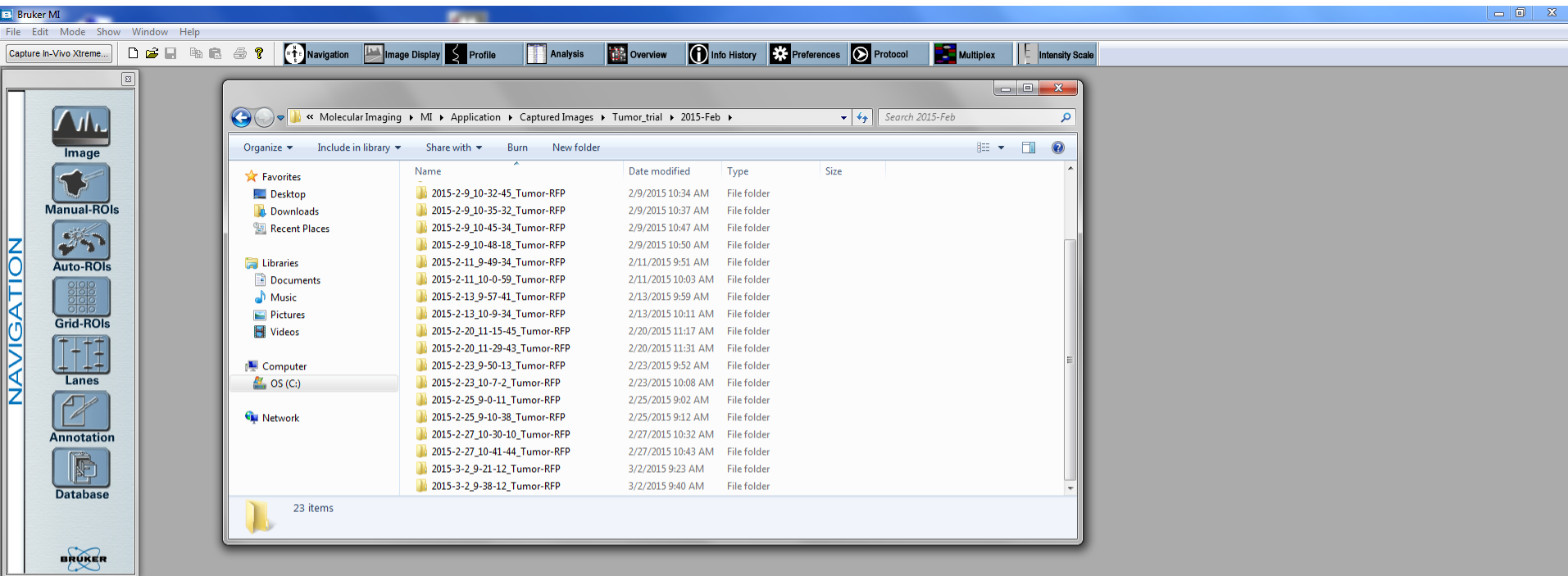


Windows Explorer Folder icon

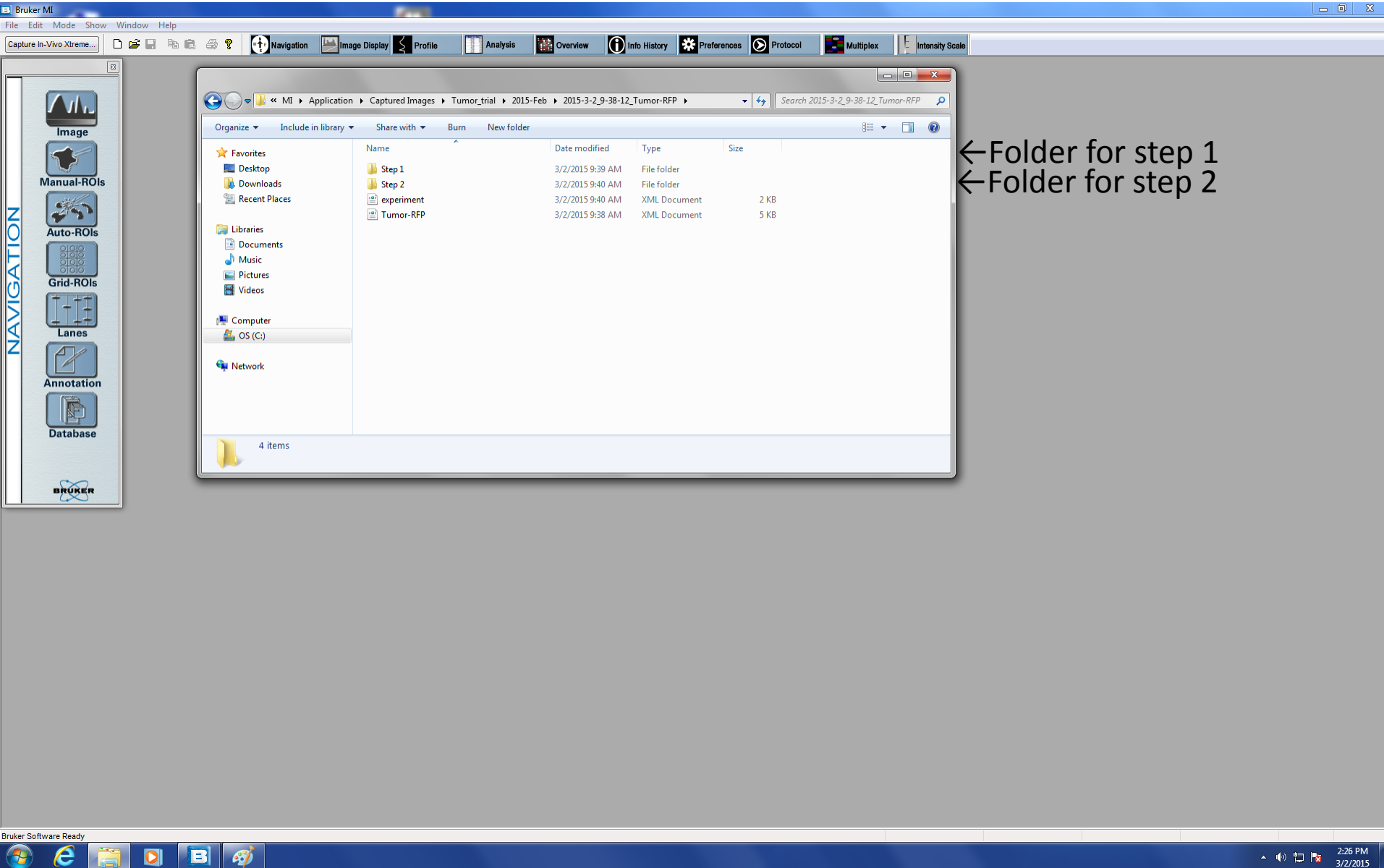
Locate the folder using the path that you used for “Saved images to”. In our case, we used “Tumor_trial”. Double click on folder.



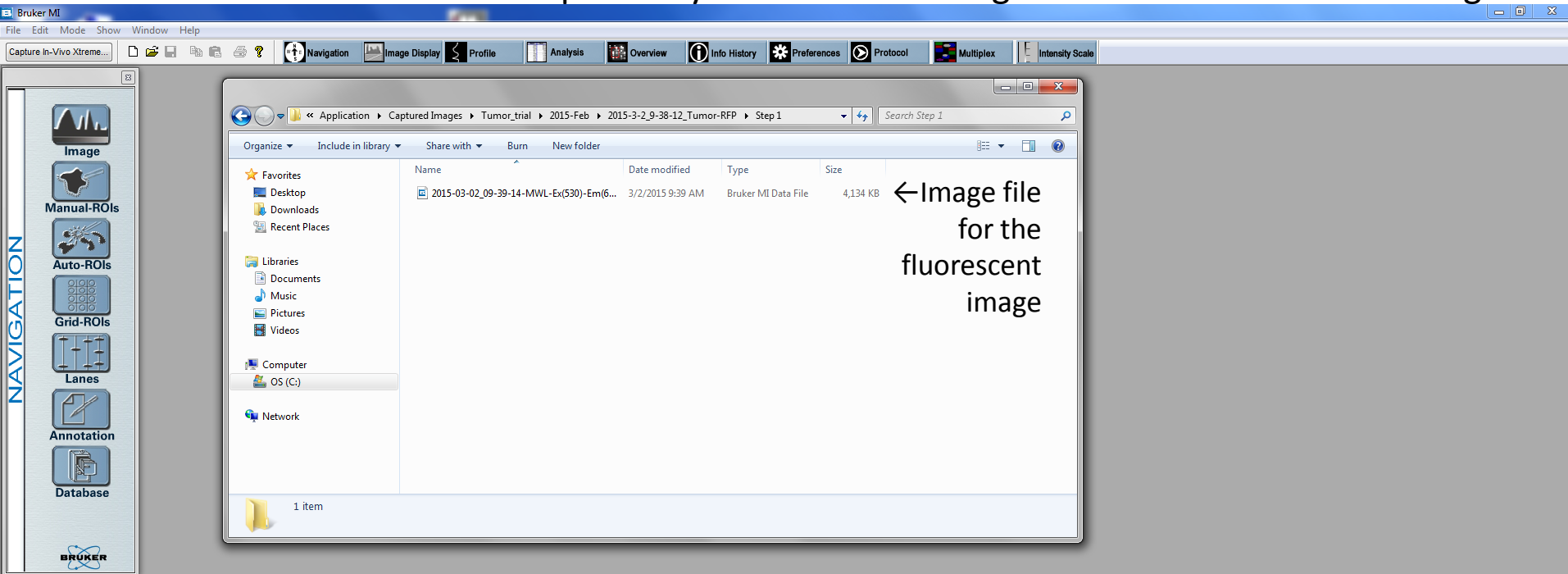
The folder contains folders that were generated with each execute command from the protocol. The folders are dated, numbered, and include the protocol used. Double click on a folder to see images in folders titled step 1 and step 2.



This is what you will see when you double click on a folder: a folder for step 1 and a folder for step 2.



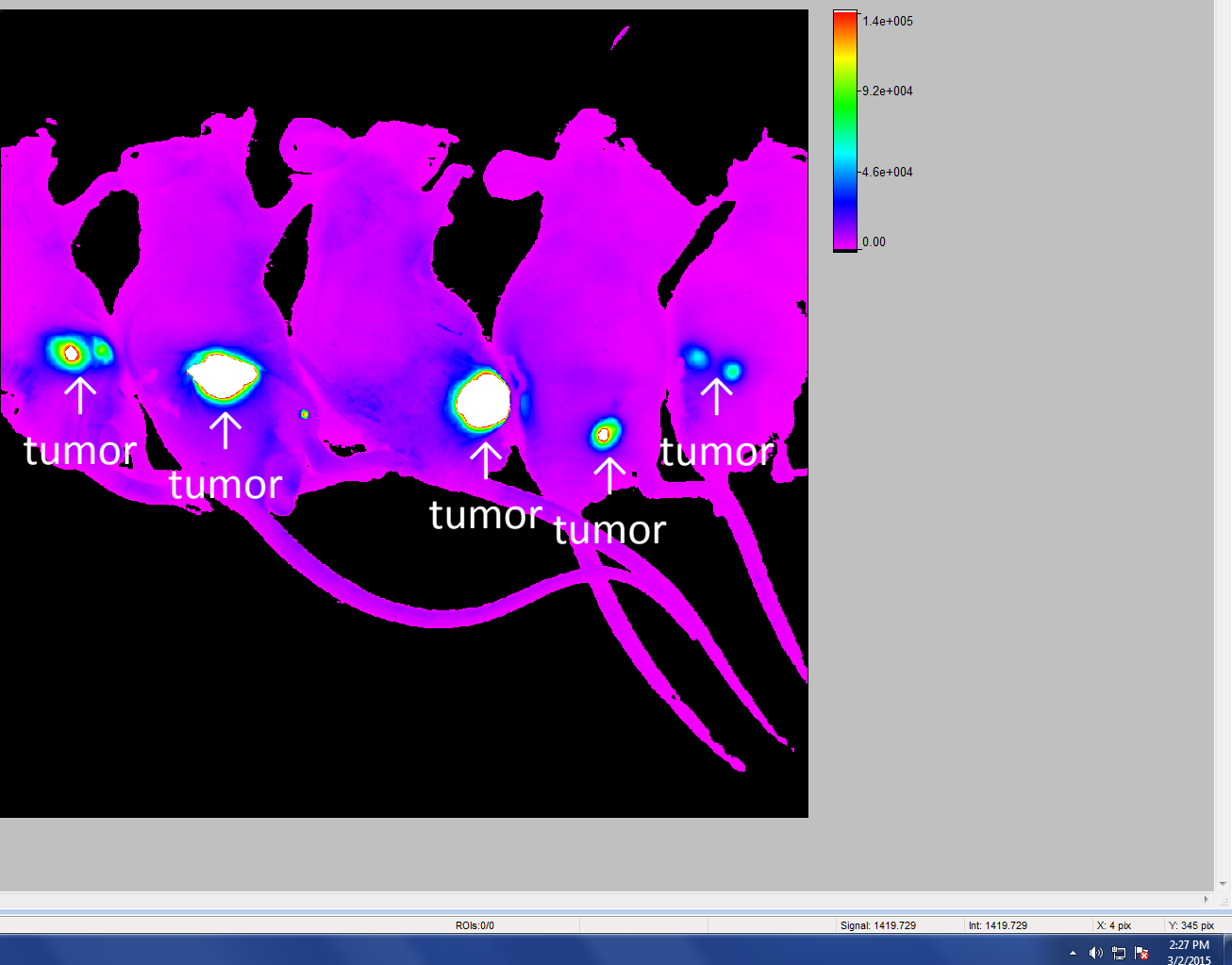
Double click on Folder for step 1 and you will see an image file for the fluorescent image.



If you double click on Folder for step 2 and you will see an image file for the XRAY image.

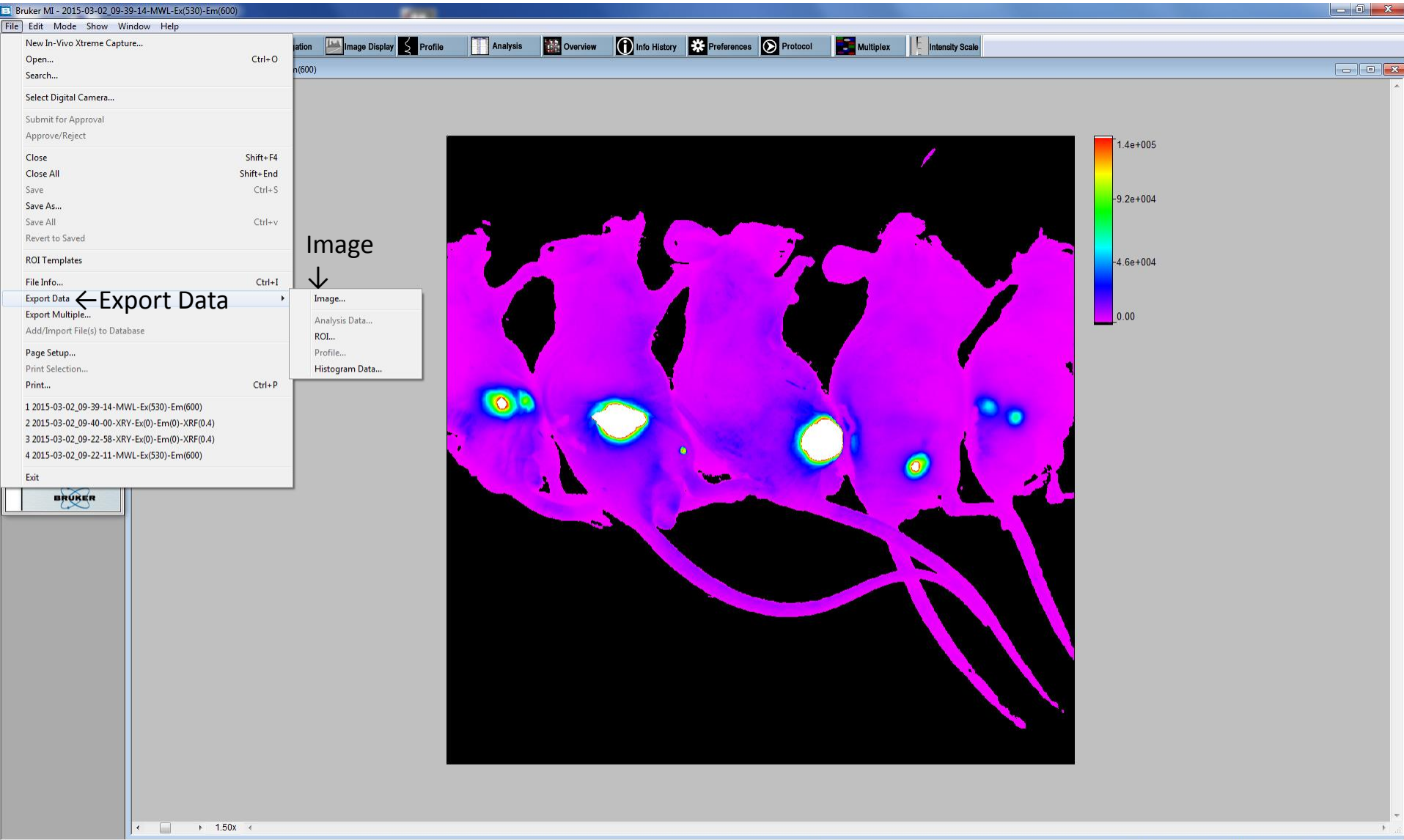


Double click on the image file; the fluorescent image will appear. The mice shown in this image have xenograft tumors from tumor cells that express red fluorescent protein.



To save the image as a jpeg file, with image on screen, click “File”. Then click “Export Data”. Scroll over to “Image” and click.

File



Now you can name and save the image as a jpeg file in the folder of your choosing.

The screenshot displays the Bruker software interface. The main window shows a heatmap image of a mouse brain with a color scale on the right ranging from 0.00 to 1.4e+005. A 'Save As' dialog box is open, showing the file name '2015-03-02_09-39-14-MWL-Ex(530)-Em(600)' and the file type 'JPEG File (*.jpg)'. The dialog box is set to save in the 'My Pictures' folder. A 'Path and Folder' label points to the 'My Pictures' folder in the dialog, and a 'File name' label points to the file name field. A 'File type' label points to the 'Save as type' dropdown.

You can do the same for the XRAY image.