**Step 1.** Load the CT image data from the folder CT_Aorta (just use the first one)

Step 2. View Image Information and DICOM Metadata

- Open the *Layer Inspector* (shortcut Ctrl-I), and select the *Info* tab (below, left)
- This lists basic information about the image, like its size, voxel dimensions, etc.
- Now select the *Metadata* tab (below, right)
- This lists all the information contained in the image header
- You can search for specific entries by typing in the *Filter* field
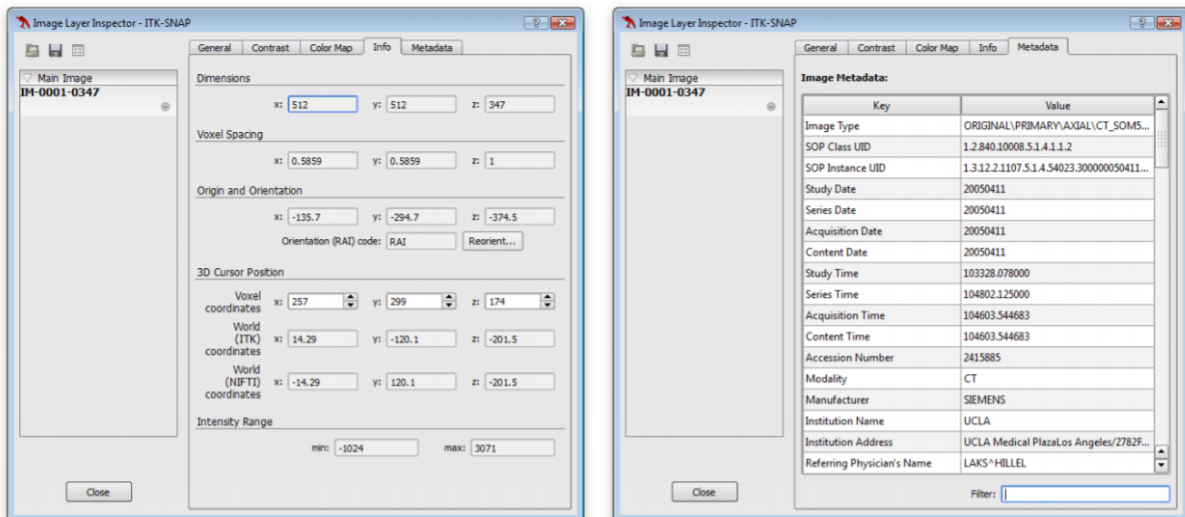
Step 2. Select Region of Interest for automatic segmentation. This is a preparatory step for semi-automatic segmentation.

- From the main menu, select Edit->View Zoom->Zoom to Fit in All Views (Ctrl-F), which will make the whole CT image visible in all three slice views
- Choose a label (i.e. colour) from *Segmentation Labels* as the active label
- Position the 3D crosshairs in the centre of the spleen (e.g., coordinates 415,320,110)
- In the Main Toolbar, select the snake-looking Active Contour Tool.

- Using the left mouse button, drag the corners of the red selection box, so that the box encompasses the spleen, as shown below
    - o Or you can type in the position (300,209,47) and size (194,216,118) of the selection box in the *Snake Inspector*, as highlighted below
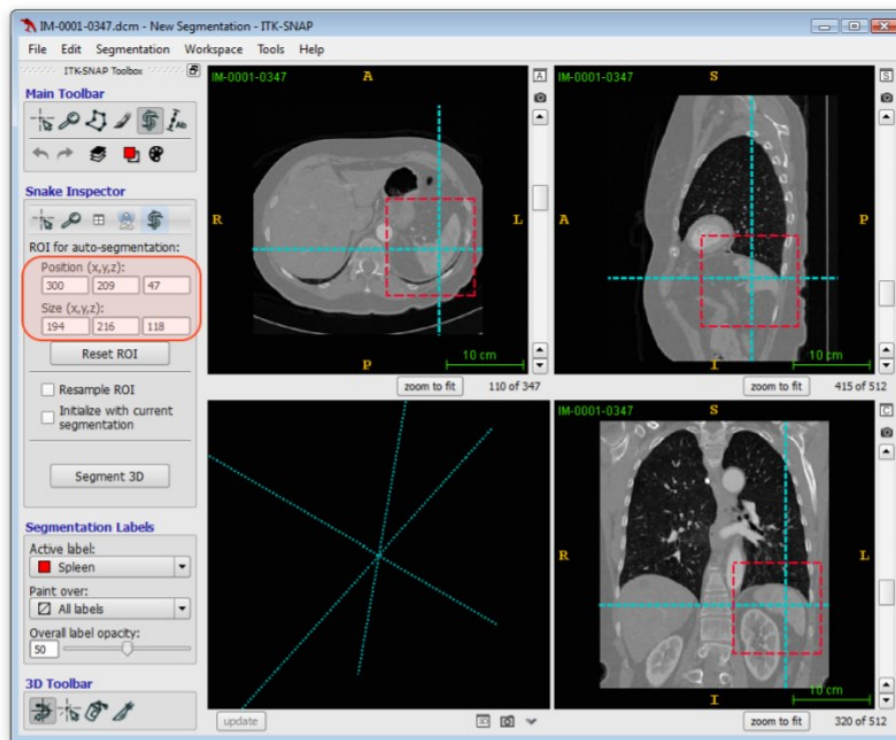
- Press *Segment 3D* to enter the semi-automatic segmentation mode.

Step 3. Use *Thresholding* to isolate the spleen

- Your ITK-SNAP window should look like the screenshot below. Each slide view will show two images side by side. On the left is the region of interest of the CT, and on the right is the so-called speed image, which should be white in spleen voxels, and blue in non-spleen voxels.

- If your images are now shown side by side, press the little button 🔲 in the right top corner of any of the three slice views

- If the CT image contrast looks wrong, select from the main menu *Tools->Image Contrast->Auto Adjust Contrast (Ctrl-J)*.
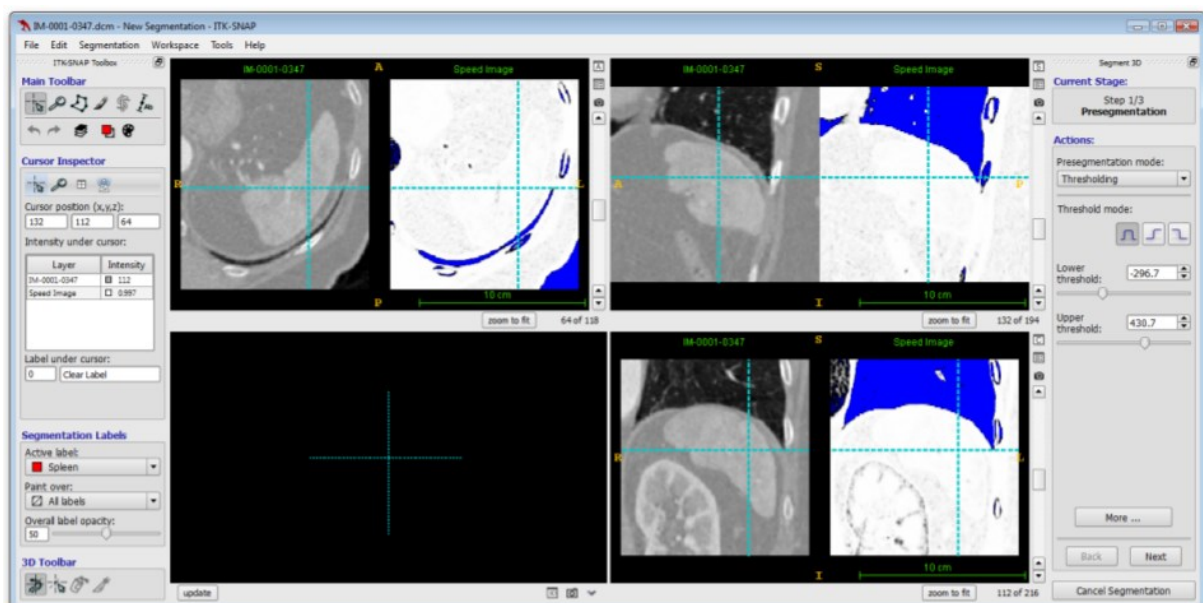
- In the tool panel on the right hand side of the ITK-SNAP window, ensure that under *Actions*, the pre-segmentation mode is set to "Thresholding"
- Use the *Lower Threshold* and *Upper Threshold* sliders to make the speed image as white as possible in the spleen and as blue as possible outside of the spleen, as shown:
  - Recommend are 67 and 237 as the threshold values
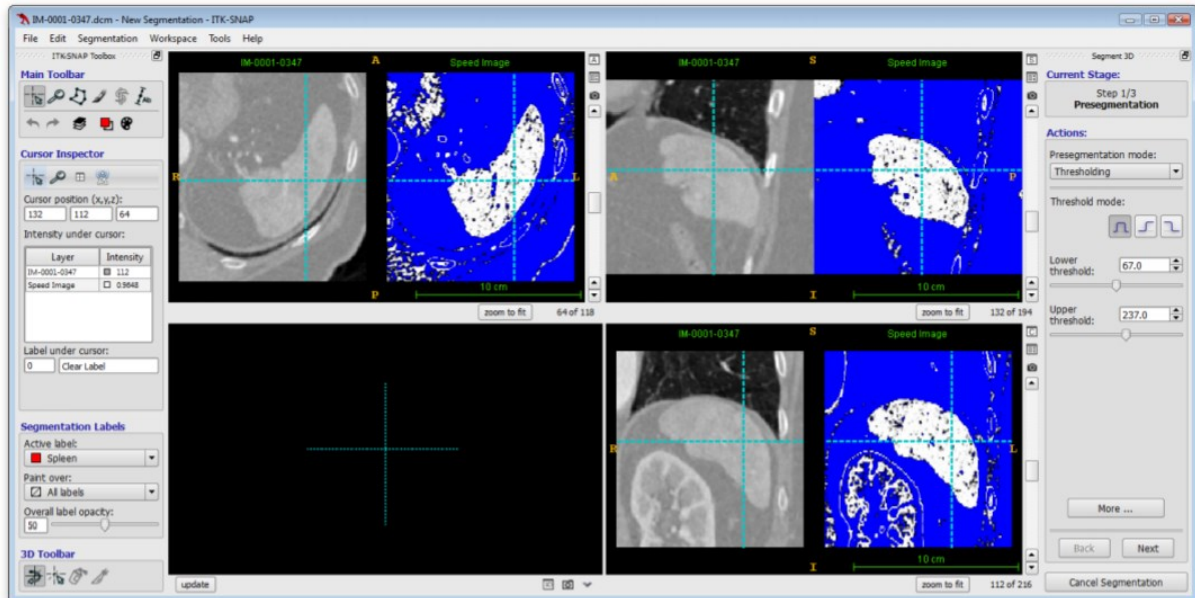
- When satisfied, press Next in the right-hand panel to continue

Step 4. Place initialization bubbles in the spleen

- You are now in "Initialization" mode, where you place bubbles to initialize the active contour.
- Position the 3D crosshair in the left anterior portion of the spleen (shown below)
- Under Actions, press *Add Bubble at Cursor* to insert an initialization bubble (shown below)
  - Modify radius of the bubble if necessary
  - By selecting the bubble you can also delete it
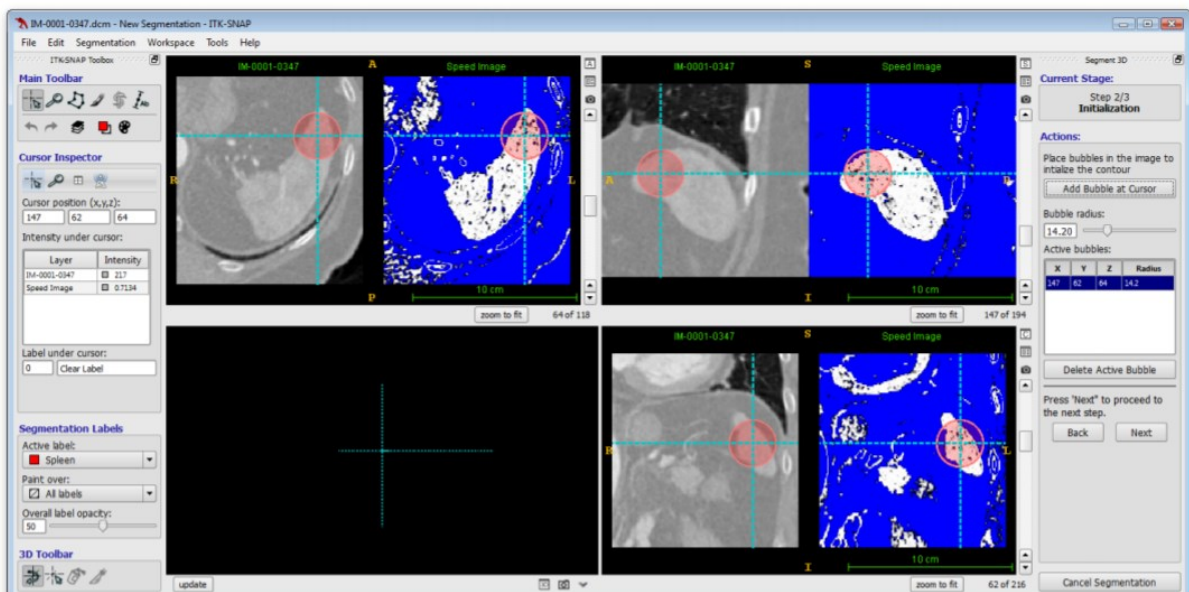
- Repeat, inserting 2-3 more bubbles in different parts of the spleen
- When ready, press *Next* to continue

**Step 5.** Run active contour segmentation

- You are now in "Evolution" mode, where you let the bubbles grow to fill the spleen
- In the 3D visualization panel (lower right quadrant), press *Update* to visualize the bubbles
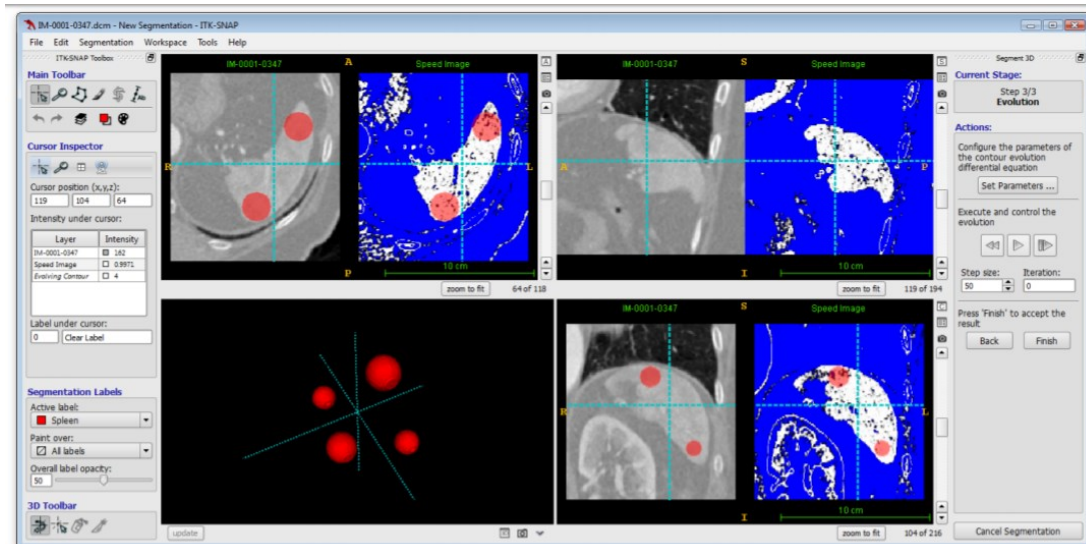

- Under *Actions*, press the *Play* button to start evolution
- As the contour evolves, you can press update in the 3D visualization panel to get a rendering
- After about 350 iterations, press the *Pause* button to stop evolution. Press *Update* to see a 3D model of the segmentation
- If you are not satisfied with the segmentation, you may press the *Back* button to return to the pre-segmentation and initialization stages, and use different threshold values or bubble placement.

Step 6. Repeat segmentation with different parameters

- To obtain a smoother spleen segmentation, we can change the parameter that controls smoothness
- Under *Actions*, press *Set Parameters* button
   o In the window that opens (see below), set a larger (0.8) value of *the Smoothing (curvature) force* (tick *Animate* to get an impression of the effect)
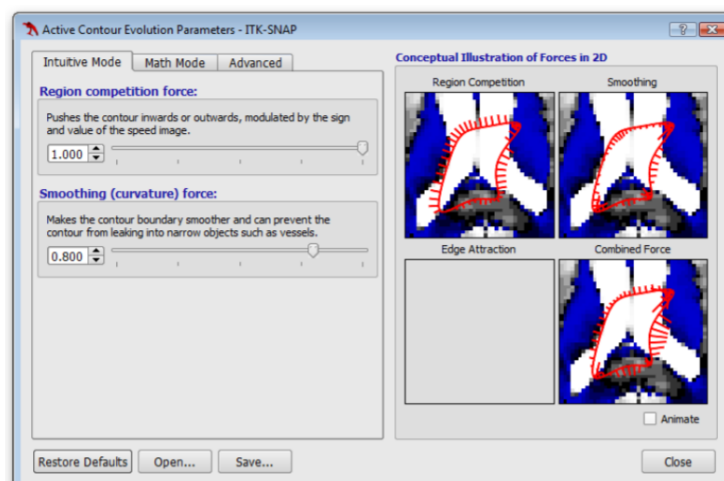
- Rewind the segmentation by pressing the *Rewind* button and run again for 350 iterations
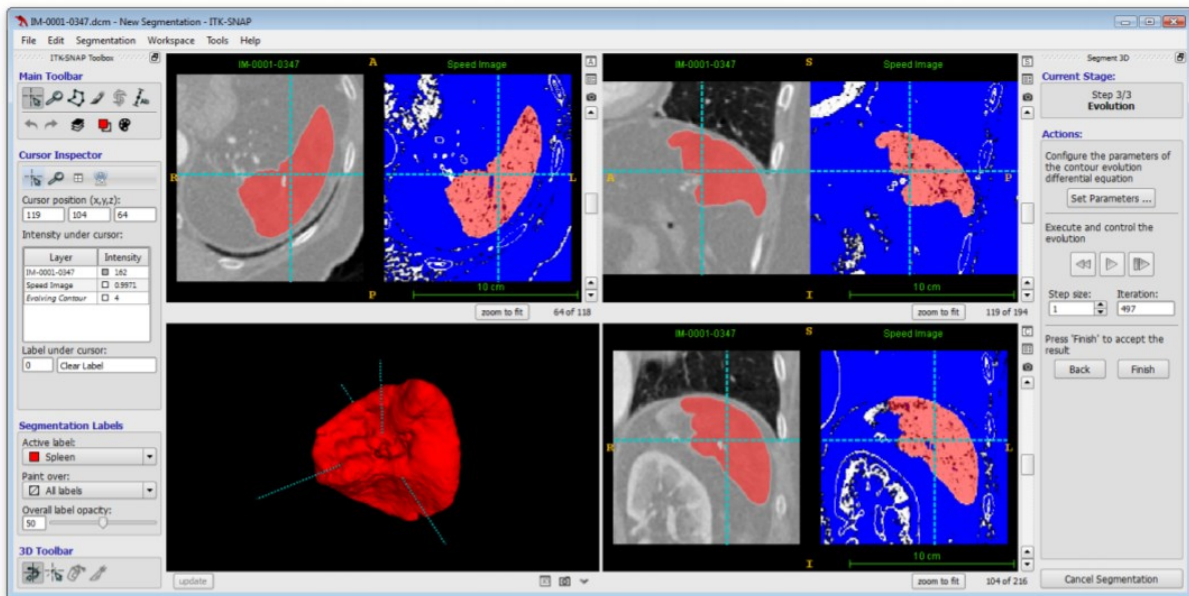
Step 7. Exit semi-automatic segmentation mode

- Under *Actions*, press the *Finish* button. Your segmentation of the spleen will be integrated into the overall segmentation of the CT image
- You can now repeat this process for various other structures in the CT scan. Choose another active label in *Segmentation Labels* before.

Step 8. Save your segmentation

- Segmentations in ITK-SNAP are saved as a special kind of image, where each voxel holds a value referencing an anatomical label
- From the main menu, select *Segmentation->Save Segmentation Image*
- Select the file format for the segmentation (NIFTI is a good choice)
- Assign a filename and hit *Finish*