

Assessment of olfactory bulb (OB) volume with „Amira“

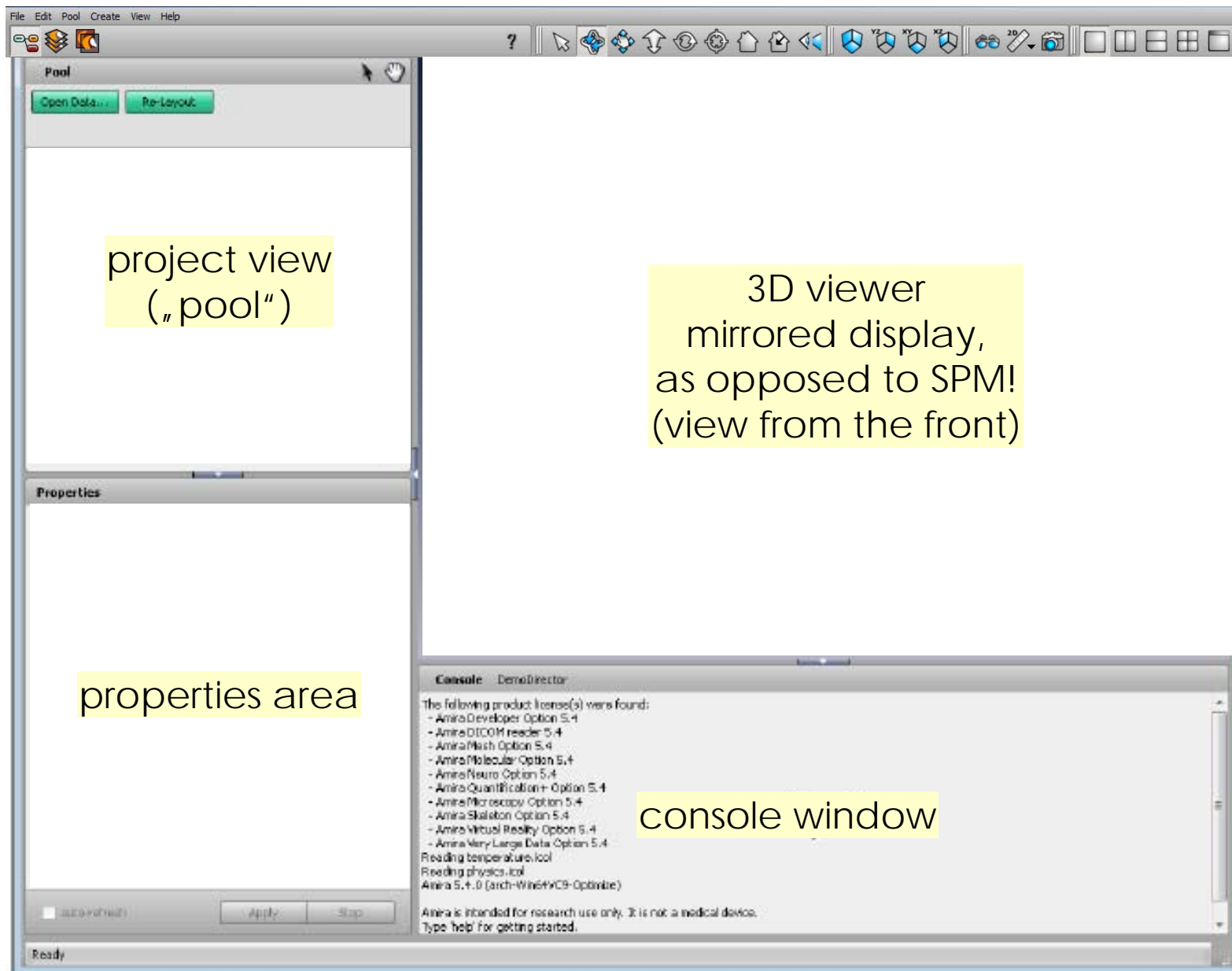
login and start procedure

- login: there used to be a common login for guests – currently, each user needs to have an individual login
- please, create a new folder for your own training results in the directory:
 - D:\Bulbusdaten\training results\
- it is suggested to create and open an XL file for tables of your results in your folder
- start program: click on amira icon



please exit amira after each patient and start afresh for the next one – the program is prone to crash when working on another data set

amira window partitions



open data

- menu File – open, or click green „open data“ button
- demo data folder:
 - D:\Bulbusdaten
- there are several folders containing data of healthy subjects „VP03“ ...
- start with VP03 for a tutorial
- click on column header „format“ to sort by format
- preselection of DICOM files
 - scroll to find DICOM files: very long filenames, extension „.ima“
 - mark all DICOM files and click „load“ (this may take a while)
- final selection of OB files
 - look for files with scanning sequence „SE“ – there should be 30 files, these are the required ones (one 3D data set containing 30 slices)
 - mark all other files - they must be removed from the list
 - using the right mouse button, select „Remove“
 - only SE files remain, now in a different display
 - mark all files and click „OK“
- the opened data set appears as a green icon („data object“) in the object pool

scanning sequence	image orientation	image number
EP\SE\EP	remove	9916014316561\... 104
SE	0.99939352505797\...	1
SE	0.99939352505797\...	2
SE	0.99939352505797\...	3
SE	0.99939352505797\...	4
SE	0.99939352505797\...	5
SE	0.99939352505797\...	6

OB files: to select only these, remove all other files from the selection

data object



data object

data object

click icon to activate data:

this will display the data's properties in the properties area
(to deactivate, click in the empty space of the object pool)



click here
to show or hide
corresponding data in the
viewer partition

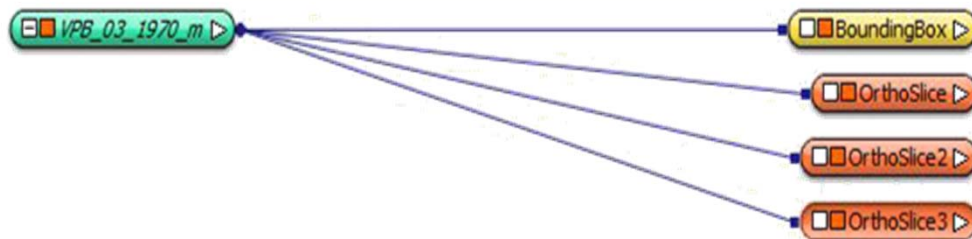
click here
to open the options menu

prepare measurement: bounding box and ortho slices

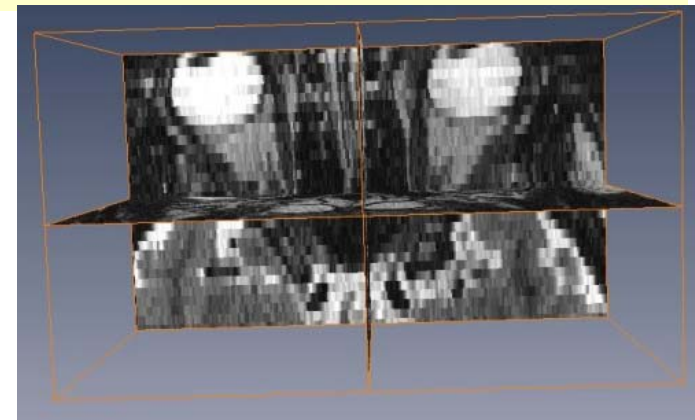


click here
to open the options menu

- **create bounding box**
- open the menu of the data object and select „bounding box“
 - a new yellow object „bounding box“ is created in the pool
 - a 3D „wire frame“ is created in the 3D view window
- **create 3 ortho (orthogonal) slices** within the 3D frame
 - open the menu of the data object and select „ortho slice“
 - a new orange object „OrthoSlice“ is created in the pool
 - in addition, a slice is created within the frame in the 3D viewer, displaying one view of the data
 - create two more ortho slices by repeating the previous step
- primarily, all slices share the same orientation; change the orientations of the second and third ortho slices
 - activate the second ortho slice (click the orange icon - note the changed appearance of the object) – the corresponding properties are displayed in the properties area
 - select (click) orientation „xz“
 - repeat the steps for the third slice and select „yz“



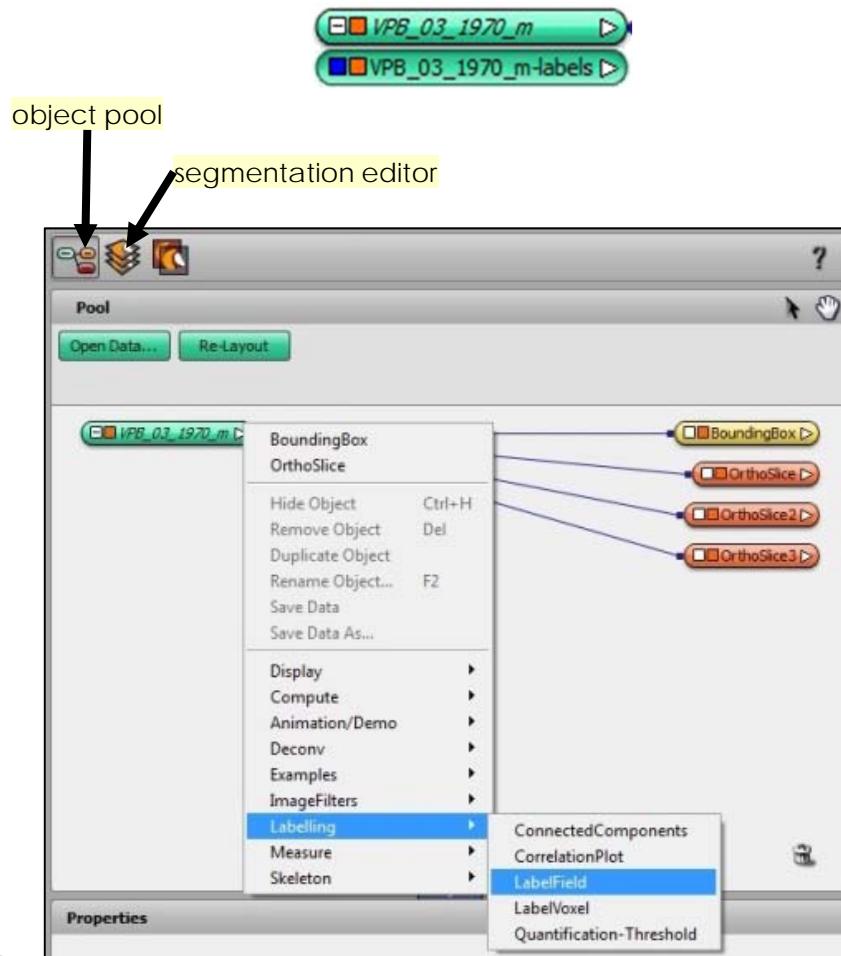
note that the activated object is displayed in a different fashion, appearing „concave“



bounding box with 3 orthogonal slices

create labels and evoke segmentation editor

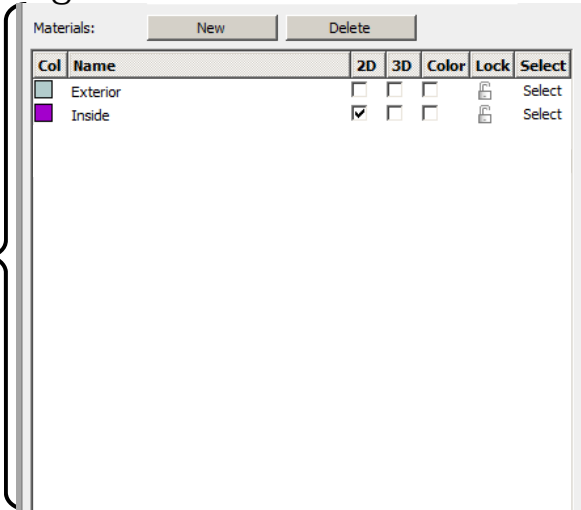
- in the menu of the data object, select „Labeling“, and further, „Labelfield“
- in the pool, a new green icon „labels“ is created
- the segmentation editor is evoked, with several panels in the left part of the window



interactive panels of the segmentation editor

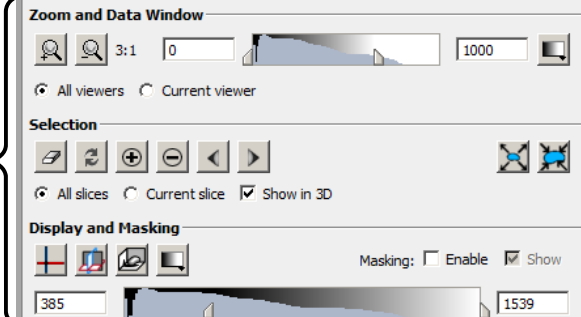
Material list:

specific parts of the image data; default are „Exterior“ and „Inside“



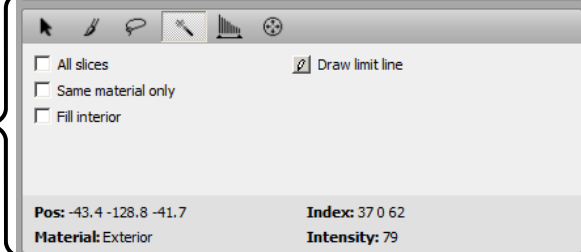
View, Selection and Display:

Below the material list several settings for viewing, voxel selection and masking are available. The settings control how the selected voxels are displayed

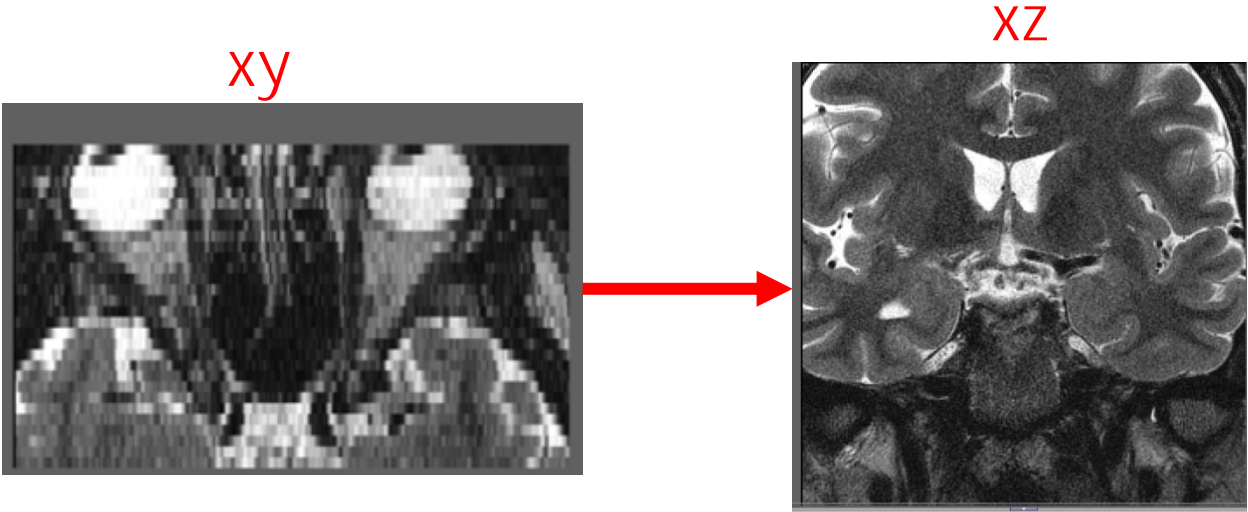
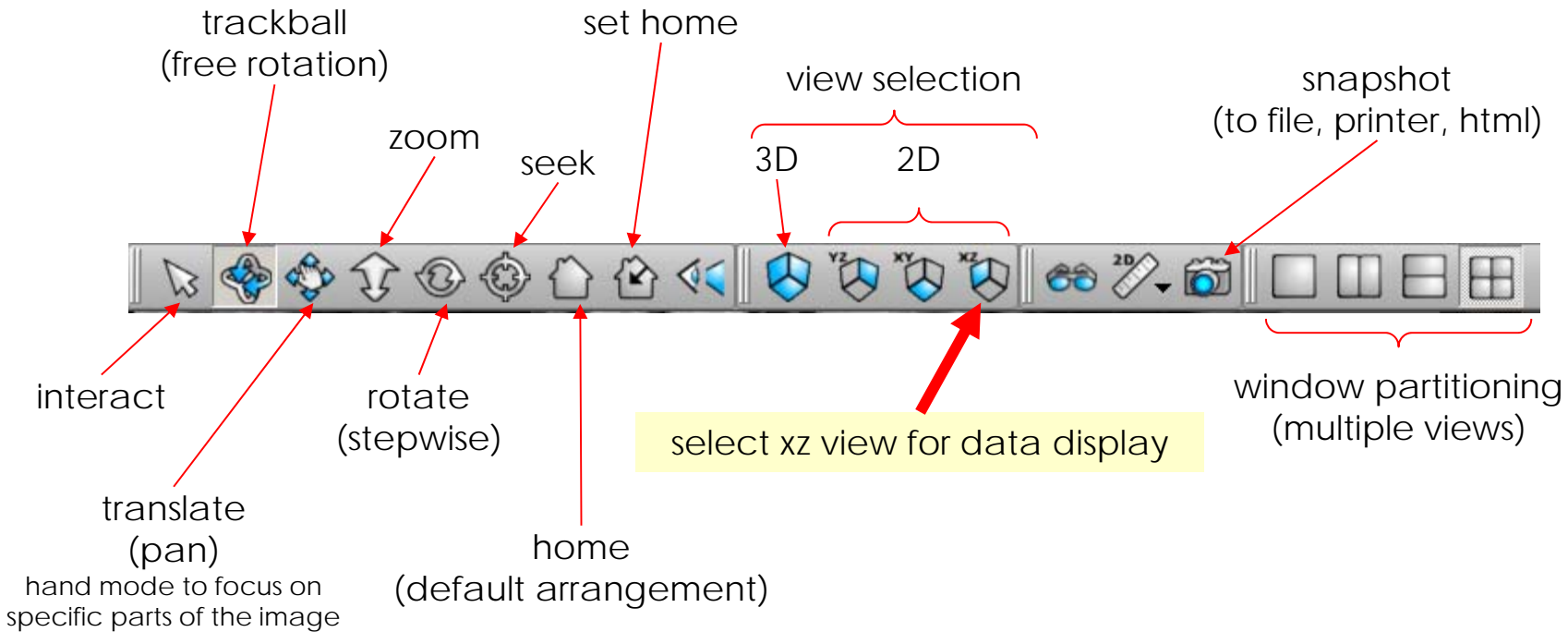


Tool Box:

Several tools for interactive manipulation of the segmentation can be selected here. Depending on the currently selected tool additional widgets show up in the options frame

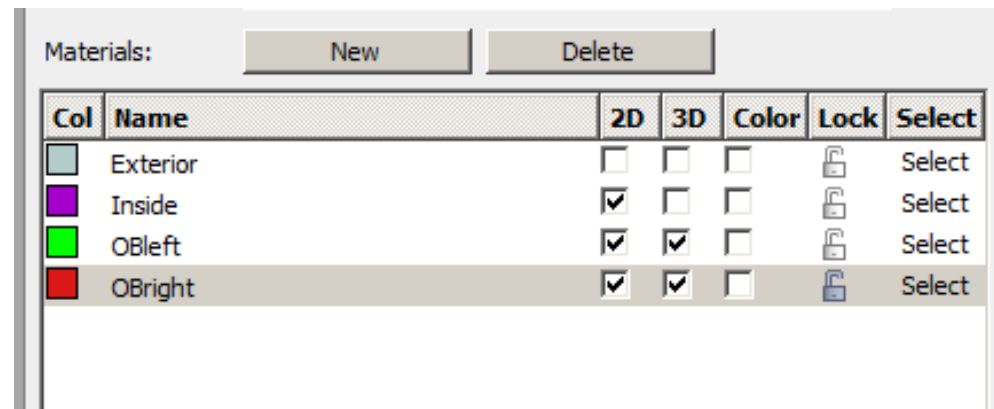


segmentation - tool bar, adjust view











create new „materials“ to represent the olfactory bulbs

- create two new materials you'll assess, namely, the olfactory bulbs in the left and right hemispheres
- click „New“ twice
- rename the new materials
 - double click the automatically created names and overwrite them, e.g. by „Obleft“ and „Obright“
- optionally, change the automatically created colours
 - click the colour boxes and select colours of your choice (note that yellow will have a poor contrast on the grayshade images)
- activate the „3D“ boxes for the new materials
- optionally, any material may be locked by clicking the lock symbol; locking prevents materials from inadvertent changes
- leave the default materials as they are, or delete them, using the delete button

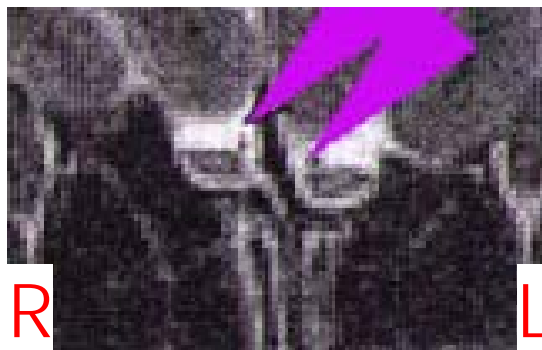


The screenshot shows a software interface with a 'Materials:' label and two buttons: 'New' and 'Delete'. Below these is a table with the following columns: Col, Name, 2D, 3D, Color, Lock, and Select. The table contains four rows of material data.

Col	Name	2D	3D	Color	Lock	Select
	Exterior	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		Select
	Inside	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		Select
	Obleft	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>		Select
	Obright	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>		Select

adjustment of data display, identification of OB

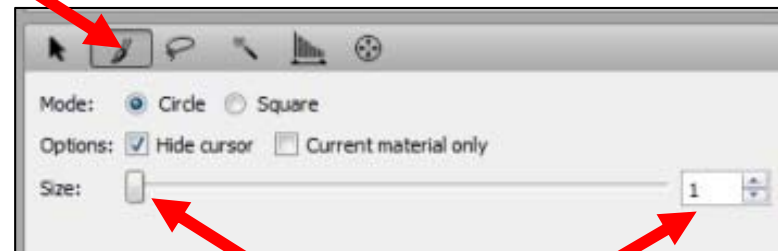
- zoom in and out
 - either use the widgets in the „zoom and data window“ panel
 - or keys:
 - zoom in: shift z
 - zoom out: z
- center zoomed view (pan)
 - click and hold the mouse wheel to shift the focus
- adjust contrast
 - use the pointer in the histogram (zoom and data window)
- scroll through the slices to identify the OB, using several zoom levels
 - use the mouse wheel to move forward and backward through the slices; current slice number and total number are displayed in the left upper corner of the image
 - identify the slices containing voxels representing olfactory bulbs
 - as numbers of slices you'll take measurements from will not be saved by amira, document the first and last slices of both OBs in your XL file
 - **basically, olfactory bulbs appear dark within a lighter fringe**
 - **occasionally, dark dots appear next to the OB which represent blood vessels (indicated by purple arrows in the example)**
- see also tool bar on earlier page



please note: the images are displayed in mirrored view – the right hemisphere is on the left side of the image and vice versa!

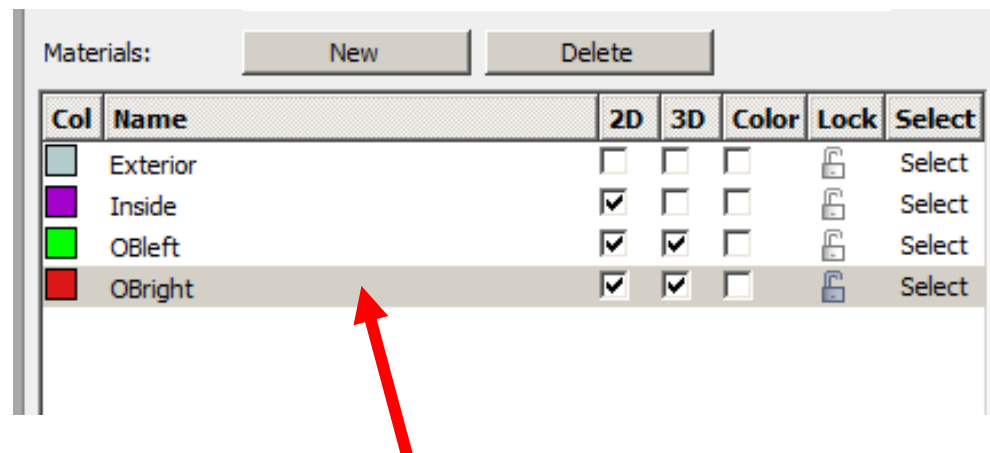
assessment of OB voxels – start









- select the brush tool and its smallest size (1)
- assess the OB in one slice on one side at a time (the sequence of slices and sides is irrelevant), performing steps 1-3 described on the subsequent pages



assessment of OB voxels – step 1: select material

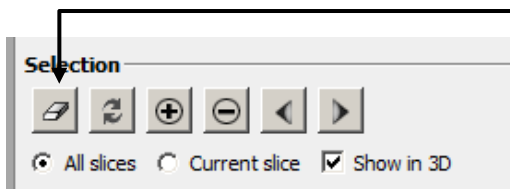
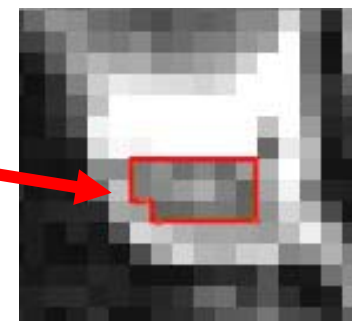
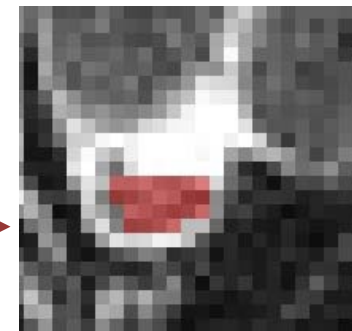
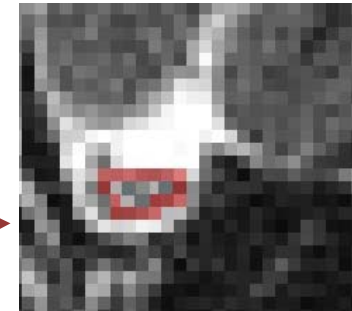
- select the material to assess: click the line of the material to mark it (the line is highlighted in gray)
- note that the brain is displayed in a mirrored view: the left hand side of the brain is on the right hand side in the display
- optionally, lock the contralateral OB material to prevent its accidental modification
- another click on a material will highlight the line in blue and enable overwriting the name!



Col	Name	2D	3D	Color	Lock	Select
	Exterior	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		Select
	Inside	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		Select
	OBleft	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>		Select
	OBright	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>		Select

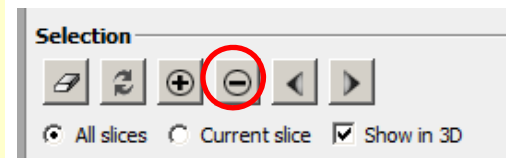
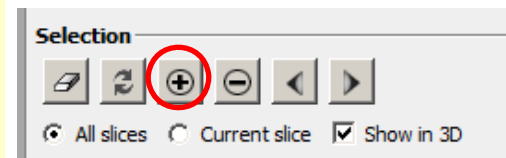
assessment of OB voxels – step 2: preselection of voxels

1. zoom in to be able to identify and click single voxels
2. mark a rough outline of OB voxels: hold down the left mouse button and move the pointer; the voxels will be marked in a brownish dark red
3. add the voxels within the outline by right clicking in this area
4. to unselect the entire marked area, click the rubber symbol in the selection panel (if the 3D toggle is activated, the selection is cleared in all slices)
5. to accept the marked area as a preliminary selection (for fine tuning, see next step), press the plus key in the keypad or click the plus button in the selection panel
 - the selected area is outlined by a fine line the color of the current material; please note the slight difference between the brownish marking color and the material color „red“



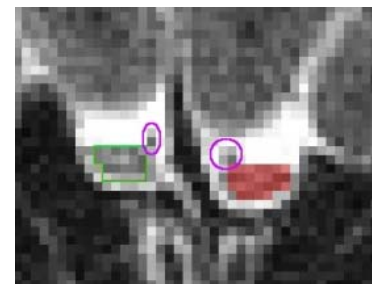
assessment of OB voxels – step 3: fine tuning of voxel selection

- to add voxels to the selection
 - click the voxels in brush mode
 - they are marked in the marking color
 - press the plus key in the keypad or click the „plus“ button in the selection panel
- to remove voxels from the selection
 - click the voxels in brush mode
 - press the minus key in the keypad or click „minus“ in the selection panel
- check the current selection, comparing it with previous ones in other slices and with what you assume to represent the OB
 - scroll back and forth between slices using the mouse wheel
 - switch between hiding and showing the material's outline, by unchecking/checking the 2D option of the current material
 - zoom in and out (shift z and z)
- you can at any time edit any selection by marking the material and adding/removing voxels



Materials: New Delete

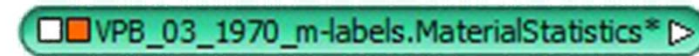
Col	Name	2D	3D	Color	Lock	Select
	Exterior	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		Select
	Inside	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		Select
	OBleft	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>		Select
	OBright	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>		Select



- green outline: selection for the right hand side olfactory bulb,
- brown area: preliminarily marked area for left hand side OB
- purple outlines indicate blood vessels (added outside amira)

calculation of OB volume

- assess OB voxels in all pertaining slices by repeating steps 1... 3
- create table of OB voxels
 - menu – Segmentation – Material statistics
 - a statistics table is displayed, showing the materials' voxel counts and volumes in mm^3
- save table
 - export table:
 - click „Export to workspace“
 - click OK in „Generate ... table?“
 - a new green icon is created in the object pool
 - to reopen the table, click the icon, and in the properties area, click „show“
 - copy to XL
 - mark, copy and paste cells to your XL table

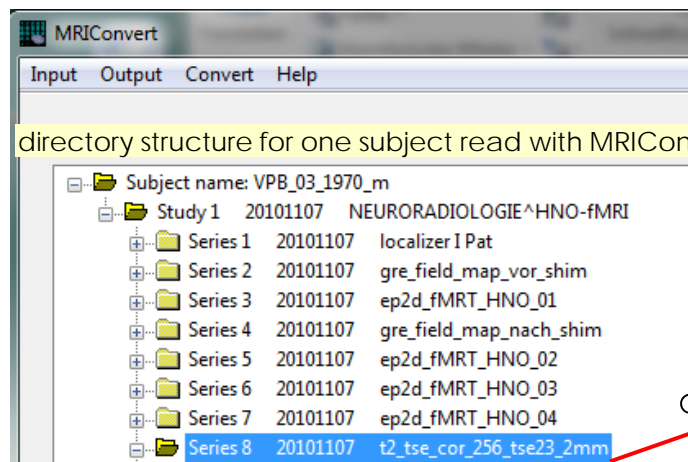
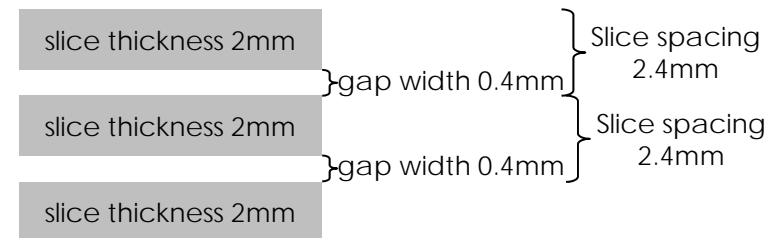
The screenshot shows a table with the following data:

	Material	Count	Volume	CenterX	CenterY	CenterZ
1 1	Exterior	1966063	1036791.1	-0.99305403	-94.042549	-11.019011
2 2	Inside	0	0	0	0	0
3 3	links	0	0	0	0	0
4 4	rects	17	8.9648438	-8.4241848	-102.44262	-4.1945038

correction of OB volume

as amira only includes slice thickness in the calculation, a correction is necessary, taking the gaps between slices into account

- determine slice thickness and gap width (in the demo data, the gap is 0.4mm, slice thickness 2mm)
 - with the free software „MRIConvert“, use „add folder“ to open the folder with the DICOM data
 - open subfolders until you reach the T2 data set
 - right click the data set, and select „Series info“
 - in the displayed information, find „SliceThickness“ and „SliceSpacing“ (in the demo data, the latter is 2.4mm)



OB series – excerpt from „Series info“

Number of averages: 4
Slice thickness (mm): 2
Slice spacing (mm): 2.4
Image columns: 256

- calculate the factor of (Slice spacing : Slice thickness); in the demo data, the factor is 1.2
- multiply the volumes calculated by amira with this factor (preferably in your XL table)

repeated OB volume measurements and comparison with standard values

- in order to improve validity, it is essential that OB volume measurements be repeated
 - either in repeated evaluations by one analyser (sessions with a given data set should not be performed in immediate succession)
 - or by different analysers
- if the two results differ by 10% or more
 - add another evaluation, then exclude the most outlying result, accepting the two results with the least difference
 - if the third result, again, shows a 10% difference, consider another MRI scan, or try to consult an expert; ultimately, the subject may have to be excluded from the study
- calculate the mean of 2 results as the final result
- compare results with the standard values published by Buschhüter et al 2008 (Buschhüter, D., Smitka, M., Puschmann, S., Gerber, J.C., Witt, M., Abolmaali, N.D., and Hummel, T. 2008. Correlation between olfactory bulb volume and olfactory function. Neuroimage. 42:498–502.)

Cut-off volume of the OB at the 10th percentile of the distribution representing the border separating normal from hypoplastic OBs, presented separately in relation to the subjects' age and sex		
	<45 years old	>45 years old
Male subjects	>59 mm ³	>52 mm ³
Female subjects	>54 mm ³	>43 mm ³
Male and female subjects	>58 mm ³	>46 mm ³

3D display of the olfactory bulbs - preparation

- create Surface View
 - switch to object pool
 - in the „... labels“ icon, open the menu (click white triangle) and select „Surface generation“
 - in the bottom window, click „Apply“
 - a new green icon „... labels surf“ is created
 - from the menu of this icon, select „Surface View“
 - a new yellow icon „SurfaceView“ is created
- make sure the 3D boxes are checked in the materials list
- do not smooth OB surfaces, the structures are too small to survive smoothing



3D display of the olfactory bulbs

- switch to the segmentation editor
- select the 4-partitions view
- in the first 3 quadrants, select the three different 2D views yz, xy, and xz
- in the lower right quadrant, select the 3D/xz view
- use the slider bars below the 2D views to shift the planes so as to allow an optimal view of the Obs in the lower right quadrant
- click the trackball icon to enable free rotation, hold the mouse key and move the mouse to rotate the 3D view
- select the single-window view to display the 3D-view in the entire window
- enable snapshot mode and take pictures, save them in your results folder
- **caution: do not use full screen mode! It gets stuck and cannot be reversed, crashing the software (if this happens, use the task manager to close amira - unsaved data are lost)!**

saving results

- in the File menu, select „save network“
- select the „automatic“ option
- save the network in your folder at D:\Bulbusdaten\training results\...
- when reopening the network, select the „script“ option

please exit amira after each patient and start afresh for the next one –
the program is prone to crash when working on another data set