VINCI MP4Aml Tool: User Documentation

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November 4, 2009

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Introduction

MP4Aml is a VINCI plugin for evaluating PET studies using the MP4A and MP4P tracers. **MP4A** and **MP4P** are shorthands for ^{11}C labeled N-methyl-4-piperidyl-acetate and -propionate. These tracers are used for in vivo estimation of activity of the enzyme Acetylcholine Esterase (AChE) in human brain. Enzyme activity is correlated to the rate constant k_3 of a 3-compartment kinetic model. From a dynamic MP4A or MP4P PET scan, the tool will compute a parametric image of k_3, and regional value tables based on averaging over voxel sets of that image.

MP4Aml has been developed from an earlier version (MP4Akin) based on previous work by G.

Zuendorf, which relies on using a fixed mask for voxel preselection and filtering. The letters *ml* stand for “maskless”: MP4Aml is distinguished from MP4Akin by not using the **Zuendorf Mask** (see [1], section 9.3.1.). Computation is attempted for every voxel of the brain, with a failrate of 5 to 20% differing from patient to patient. Various other policy changes have been adopted, including a novel algorithm based on weighted nonlinear least squares fitting (NLS). Full background information on the current version is given in [1], which is used as a reference throughout this documentation.

Version

This documentation refers to the second release of MP4Aml. Its main difference to the first version is that two default settings have been changed in response to the findings of chapters 14 to 16 of [1]: ‘**padding**’ has been changed from 2 to 3, and ‘**regionsize**’ from 674 to 1557 (see section 5.4). Apart from these changes, it is computationally equal to the first release.

To find out if you are running the second release, proceed as follows: Open ”Info View” in the toolbar of an ortho display. In its window, check ”Configuration” which is a subitem of ”About”. This brings up a list of ”enabled plugins” where you can look for ”MP4A maskless” and its release date, which should be November 2009.

1 Getting started

This chapter contains what is needed to compute the first parametric images.

1.1 The User Interface

MP4Aml is invoked by selecting “MP4A maskless” from the Tools menu of an orthodisplay.

Be sure to avoid “MP4A kinetics”, it refers to an older version !

In its top section, MP4Aml has four edit fields for specifying input filenames. Underneath, there is a **Middle Section** groupbox containing 5 buttons and an “# Early frames” and a “Stop before Step” selector. Ahead of the buttons, an explanatory note (**”Explain Text”**) and a **”Prompt Text”**are being shown, the latter in bold face. Under the Middle Section, there are fields for making choices on the computed images. At the bottom row, there is a “**Regional**” button for computing regional statistics, followed by a **Status Area** for showing status messages. It may not be visible upon startup.

1.2 Parametric Images

MP4Aml computes parametric images of q_1, k_2, k_3, *k3dev*, *k3var* and a few experimental image modalities. k_1, k_2, k_3 are kinetic constants controlling transport and degradation of the MP4A tracer. q_1 is the quotient $\frac{k_1}{k_{1r}}$, which is k_1 in the tissue of interest divided by k_1 of the reference region. *k3dev* and *k3var* are voxel based error estimates as discussed in [1], chapter 8. *k3dev* estimates the standard error of every computed k_3 based on the residual squares of its nonlinear fit.

1.3 Automatic Runs

Upon installation, the software is configured to compute a k_3 and a *k3dev* image once you enter a valid input file name to the top selection field, hit the <Run> button of the middle section and walk away. It will be busy with normalization and coregistrations for the next twenty minutes, followed by shorter timespans of image preprocessing and kinetic analysis. If the “auto save all” checkbox above the Regional button is checked, the resulting images will be written to the patient directory automatically, using the interfile format.

The plugin automatizes a complex workflow of 6 steps. In case anything goes wrong, user intervention may be required. It is therefore possible to run the steps individually.

1.4 Navigating Steps and Phases

MP4Aml takes raw input data from a PET scanner and performs normalization, coregistration, Gaussian filtering, before computing the parametric image(s). It comes down to a linear succession of 6 steps, numbered 0 to 5.

The current step is displayed in the groupbox title of the Middle Section groupbox. During computation, it will say “Step XX in progress”, during halts: “Ready for Step XX” or “Step XX finished”.

The text underneath says what the current step is about, and which inputs and interactions are expected from the user.

To stop in the "finished" phase of a step, set the "Stop before Step" selector to the following step. Setting it to 1 upon startup, will force execution to stop in the "finished" phase of step 0. From there, click <Run> to proceed to the "Ready" phase of step 1. Its only effect is to see the prompts change, there is no action performed, and one can always navigate back using the <Back> button. To proceed through step 1 only, set the "Stop before Step" selector to 2 before clicking <Run> again. Step 1 computes what we call the "Flow Image", adding up the scan frames of the first 10 minutes p.i. without coregistration, and displays it in a VINCI image buffer. Navigating <Back> from the "finished" phase to the "Ready" phase of a step will undo all computations. In the case of step 1, the image will be cleared.

1.5 Settings

Image computation is controlled by a large number of settings. Major settings are edited via selectors on the main widget, they are documented in this section. Minor ones are more numerous and hidden in the **<Settings> widget** that opens when clicking the <Settings> button. They are grouped by the step during which the setting is active, or to which it relates.

During halts, editing is enabled for all settings referring to future steps, disabled for settings referring to the past. There are two exceptions: the **"Early Frames"** selector above the <Run> button, and the **<skip>** selector further down. By the standard policy, "Early Frames" should cover the first 10 minutes of scantime, and the frames of the first single minute should be skipped. Other choices are discouraged. The fields are therefore disabled until MP4Aml has read the framing times of the input file (during step 0) and computed suitable defaults. If you do want to change these settings, stop before step 1.

The **NLS checkbox** allows to chose between voxel based NLS, which is a weighted Nonlinear Least Squares Fit ([1], chapters 4 and 5) and the COLOGNE method that was the method used in MP4Akin (chapter 3). **"Gaussian FWHM"** is the filter width applied during image preprocessing before voxel based activity curves are sampled.

By clicking the <**Modify**> button of the main widget, you can select the image modalities computed during step 5. Sensible choices are only q_1, k_2, k_3, *k3dev* and *k3var*, where the latter two are not available for the COLOGNE method. The rest are experimental modalities.

1.6 Settings Report

All settings affecting the output, except "skip" and "Early Frames", are remembered between the sessions. This makes it easy to apply identical procedures to every patient of a group. It creates, however, the risk that forgotten settings could spoil results. As a safeguard against involuntary changes, the following features are provided:

- **Startup Message:** The number of settings differing from default is indicated in the Status Area after startup. If the number is zero, there is no message.
- <Report> button of the <Settings> window: Can be clicked at any time to count the number of settings differing from default, and print a report to the VINCI log. The report contains a brief description of every setting, its current value and default, and what you need to do to regain the default value.
- <Native> button of the <Settings> window: Resets all settings, including those of the main window, to their defaults. The button is disabled after steps 4 and 5 in order to prevent it from changing settings of the past.

If, after making changes, you want to regain the settings of the previous session, close the plugin with the <Quit> button. Unlike the standard close button, <Quit> does not save anything to disk, so the current settings will be "forgotten".

1.7 In-Depth Description of the Steps

Step 0: Validation of the Input Data

At the beginning of step 0, the plugin expects the names of 4 input files. They will be called **Input Item 1** to **Input Item 4** in the following, by the order of the input fields. Input Item 1 is an ECAT

7 multiframe PET image file holding the data of the original acquisition. In its header, it carries information on the patient, the scanner and its settings, the image format and the timescale used for the frames. Part of this information is extracted from the file in step 0 and stored internally. It can later be accessed with the <Results> button (see section 3 ff). The other Input Items refer to files that are part of this distribution. They reside in the templates/mp4a2 subdirectory of your VINCI installation. The nature of these files is explained in section 4. All Input Items are checked for their existence as files. Items 2 to 4 are loaded to VINCI image buffers. To render them visible, use the <Results> button.

Step 0 is ready to run once the four input fields contain valid entries. Fields 2 to 4 should contain valid entries upon startup. In field 3, you have the choice between different mask precursor files (see section 1.7, step 4b). Fields 2 and 4 are without alternatives, they contain the files PET1.v and fullbrainmask1.v.

Step 1: Computing the Flow Image
The Flow Image is the decay weighted sum ([1], section 2.7.2) of as many early frames as the "**Early Frames**" selector indicates. The default is the number of frames that cover the first 10 minutes of scantime. It is computed during step 0, then the selector is activated to allow changes by the user. The Flow Image is used in steps 2 and 3 for normalization/coregistration. It has better statistics than individual frames, which tend to be thin in PET multiframe studies. If the patient didn't move during the first 10 minutes, the Flow Image should be sharp enough to allow good coregistration results.

Step 2: Computing Normalization Parameters
All frames must be normalized to the Normalization Template. This is brought about by an affine mapping defined by 12 scalar parameters. They are computed only once: by running "affine normalization" of the Flow Image, using VINCI's "Multi Modal Matching-" or **MMM-Tool**. The resulting set of mapping parameters is used in step 3.

During this computation, The MMM tool will open automatically and you can watch the registration both on its progress display and in the VINCI logfile. The MMM tool is controlled by an XML script. For users familiar with this interface, the script is available for editing (<Settings>, step 2).

Step 3: Correction for Movement and Computing Normalized Frames
Since we assume there has been no patient movement during the first 10 minutes, the "Early Frames" are already coregistered with the Flow Image. So for them, normalization comes down to applying the mapping of the parameter set obtained in step 2. The remaining "Late Frames" are, in addition, corrected for patient movement. This is achieved by separate coregistration of each frame to the Flow Image, then applying the step 2 mapping in the same way as with the "early frames". For a more precise description of this part of the workflow, see [1], chapter 9.

The coregistrations make step 3 the most time consuming step of the workflow. They are "rigid body" coregistrations having 6 degrees of freedom, compared to the 12 required for "affine normalization" of step 2. The output of step 3 is a set of "Normalized Frames" which are congruent with each other and the Normalization Template and share its volume and pixel size. They are written to disk in an internal file format. Should there be several passes through step 3, as a result of user intervention, these files will be used again during every later pass.

Like in step 2, the coregistrations are controlled by an XML script, which is available for editing (<Settings>, step 3).

Step 4a: Extracting the Reference Curve
During step 4, the reference curve is extracted from the unfiltered normalized frames. It is a table containing one value per frame. Every value is the average of a set of voxels of its frame, which are identified by a so called **reference mask**. That mask is generated in adaptation to the patient anatomy by a method described in [1], sections 10.2 and 10.3.

Mask generation relies on a precursor mask which determines the reference region to be used: Putamen, Cerebellum or the right or left half of either Putamen or Cerebellum. The precursor mask is read from either a textfile or an interfile. The default is “putamen1.txt”, the rest are enumerated in section 4.4.

Besides the precursor mask, which is selected by browsing for input item 3, mask generation is also controlled by parameters ‘**padding**’ and ‘**regionsize**’ (see [1], section 10.3). Both the final mask and the reference curve can be inspected after step 4 using the Results List, see sections 3.1.11 and 3.1.12.

Step 4b: Masking and Filtering
The frames must undergo smoothing prior to extracting voxel based activity curves in order to reduce noise. A Gaussian filter is therefore applied to every frame. Its width “FWHM” (=Full Width at Half Maximum) is the distance of the two abscissae where the Gaussian curve takes half its maximum value.

During filtering, low activity regions are protected from influx of neighboring high activity regions. This is achieved using a set of 10 to 15 masks which are generated from the image data in adaptation to the patient anatomy. Methods and policies of mask generation and application are described in detail in [1], section 10.4 ff. The process is governed by parameters **’margin’**, **’qzoning’**, **FWHM** and **FWHM3**. Their function is explained in [1], section 10.4.3. Recommended choices of these parameters are 1, 0.85, 8 and 8. Section 10.6 ff. of [1] describes how these defaults have been obtained.

Step 5: Computing Images
The “Compute what” group displays the list of image modalities to be computed during step 5. Its contents can be changed with the <Modify> button. Main choices are q_1, k_2, k_3, *k3dev* and *k3var*, the rest are experimental. *k3dev* and *k3var* are error estimates, expected to indicate the error of k_3 in units of standard deviation and variance. After making these selections, click <Run>. Progress will be displayed in the Status Area, followed by a message indicating which VINCI image buffers contain the results. It also displays the number of voxels whose evaluation failed.

1.8 Customizing k_3 Output

1.8.1 Failure and Failmarking

Conditions have been defined under which a voxel “**fails**” to evaluate. For NLS, failure occurs if convergence is not reached within a given number of iterations. That number and the steplength thresholds by which “convergence” is reckognized, can be edited in the <Settings>/step 5 menue, “max iterations”, “underway” and “final” breakoff step length. The COLOGNE algorithm decides on “failure” if unreasonable intermediate results occur. k_3 Confinement (following section) can be used to define additional failure conditions.

Failed voxels are, by default, marked with zeroes in the parametric images. For regional statistics, it is important that they be distinguishable from voxels whose k_3 is zero. With default settings, this condition is met, since q_1, k_2 and k_3 will not evaluate to a clean zero.

One may decide to use a different failmarker. This can be set in the *upper* edit line of the Failmarking group of the “Regional” panel of the <Settings> menu. It becomes effective upon *writing* new parametric images. During <Regional> statistics, the failmarker is read. It is then compared to the entry of the *lower* edit line of the Failmarking group. When doing regional statistics on your own computed data, you should therefore have the same marker in both lines.

Note that the only sensible alternative to zero as a failmarker, are negative integers. This is because voxels are stored as IEEE floats, and later compared without error tolerance.

1.8.2 k_3 Confinement

Like the previous feature, this one has been added in order to accommodate different policies that might be preferred by some researchers. By default, the range of k_3 is not bounded (except for a lower bound of zero which is a consequence of the NLS default settings, in particular of lambda=0.9). k_3 output values may exceed the highest sensibly measurable k_3, which is around 0.4, by orders of magnitude.

Such outliers will affect certain types of statistics severely, for instance, they usually spoil image/image correlations. It is therefore possible to impose mandatory confinement borders to k_3

output. Check the "apply" checkbox of the "k_3 Confinement" group of the <Settings>/Regional menue, and input your confinement borders below. The defaults are 1 and 0.000001, the latter with the idea to keep the values safely away from zero. If the "treat violation as failure" checkbox is checked, any voxel outside the confinement interval will be fail-marked (section above). If the box is not checked, the confinement borders act as upper and lower limits. Every k_3 exceeding the upper (lower) border, will then be set to the upper (lower) border.

1.8.3 Vi-Inspector

Use another small VINCI plugin called the **Vi-Inspector** to check the value range of your parametric images. It provides a list of all voxels of an image and their values. When the list first appears, it is sorted by voxel address. To resort by voxel value, hit the <s> key while the list is visible.

2 Regional Statistics

This feature allows computing statistics on atlas regions. The two atlases distributed with this release are called **atlas1.img** and **atlas2.img** (interfile format) and found in the template/mp4a2 subdirectory. atlas1 has been downloaded from MNI and resliced to Evaluation Format. It does not subdivide anatomical regions in their left and right hemispheral parts, a shortcoming that has been fixed in atlas2. The atlases must be used with descriptor files called **regions1.txt** (for atlas1) and **regions2.txt** (for atlas2). Be sure not to mix this up. Atlas and descriptor files can be selected using the respective edit fields or browse buttons in the "Regional" section of the <Settings> menu.

2.1 Getting started

Click the <Regional> button at the end of step 5. It brings up a small widget containing the list of requested table columns. Use the vertical arrow keys of the keyboard for moving the blue bar and selecting an item.

The first 4 columns are standardized, they contain the atlas key of a region, its name, number of voxels and yield, which is the percentage of voxels that evaluated successfully. Their order cannot be changed, but you can suppress the printout of each column by moving the bar to it and hitting the <Del> button. It should say "NOT INCLUDED" in the display.

Upon startup, the list may or may not contain additional lines, referring to image volumes. Such lines look like:

```
8                    k3 md
9                 k3dev   s
```

The leading number refers to the VINCI image buffer containing the volume. It is followed by the image title as indicated in VINCI. The line is concluded with three flags m,d and s, where at least one must be activated. Each flag defines a table column. 'm' computes the average of the voxels of a region, 'd' their standard deviation, 's' is the square root of the average of the squared voxel values, which is the recommended summation mode for *k3dev*. The flags can be toggled by moving the bar to the respective line and pressing <m>, <d> or <s>.

To add an image volume to the list, press <Insert> and input the corresponding VINCI image buffer number. For convenience, it is also possible to input the image modality of a newly computed image, such as q_1, *k3var* etc.. That image will be found if it has been computed in the latest pass through step 5 and if its name is not duplicated by some other VINCI imagebuffer. To delete a volume, press <Del> on it. To change their order, delete them and add them again in the desired order.

Having thus completed volume selection and chosen the right flags for each volume, press <Return>. Progress will be displayed in the Status Area of the main window, followed by a message indicating that a textfile has been printed to the patient directory. It contains the table that was specified, with the entries of every line separated by tabs, a format that is easily copy/pasted to other applications. Above the table is a header section intended for documentation, listing all evaluation settings, prefixed by unambigious interfile header keys.

2.2 Defining Table Rows

While table columns are specified at runtime as explained in the preceding section, the rows are defined in the atlas descriptor files **regions1.txt** and **regions2.txt**. These are both machine and human readable text files. If you need a special order or subset of the table rows, create a copy of regions1.txt or regions2.txt (depending on which atlas is used) under a different name, and modify it according to your needs. The easiest modification is by deleting unwanted rows. You can define new regions which are unions of several atlas regions, see for instance the definition of "Frontal" in regions1.txt or "Total Frontal" in regions2.txt. The syntax is explained in the comment sections of either file. Within the <Regional> feature, there is limited support (in the form of error messages) for debugging your custom descriptor files. Make sure they are selected before clicking <Regional>. For selection, use the second <Browse> button of the <Settings>/Regional menu.

2.3 Masked Statistics

It is possible to run regional statistics on a subset of the brain's voxels. That subset can be defined by a binary mask or a so called "**Intensity File**". Only voxels whose intensities lie between two given thresholds will be included in the statistics.

The Intensity File or binary mask must be specified in item "**Intensity File**" of the "Optional Masking" group of the <Settings>/Regional menu. The feature is activated as soon as this edit field contains a file name, and deactivated by clearing the name from the edit field. The thresholds must be specified underneath. If a binary mask is used, they can be set to, say, 0.5 and 2, which includes all voxels that are 1 in the mask and excludes all that are 0.

After making these entries to the "Optional Masking" group, work with the <Regional> feature as usual. A couple of extra lines will be added to the header section of the output file, in order to document which intensity file and thresholds were used. You will notice changes in the voxel numbers: Counted voxels are those in the intersection of the mask with the atlas regions. The "yield" column is computed with reference to this subset.

3 The Results List

is used for inspection of intermediate results compiled during steps 0 to 5. It also provides a quick way of deleting the corresponding files. Click the <Results> button to bring up the list. It can be operated with the keyboard only. (It may require a single mouseclick though upfront, to get activated and receive the keyboard focus). To navigate the list, use the vertical arrow keys, and the <PgUp>, <PgDn>, <Home> and <End> keys.

Every line refers to one "result", which is either a file or some data being held in memory. Three columns are being displayed. The right column tells which step has created a result. The middle column holds a short description. For results corresponding to files, the left column has the filename ending. This may be merely an extension (such as the ".v" of ECAT 7 image files) or a sequence of characters appended to the **basename**, which is the name of the input file minus the ".v" extension. So if the input filename is PATIENT17_amp.v, all intermediate files will be named "PATIENT17_amp" concatenated with what you see in column 1. Below the list, there are a few lines of display area for data that will fit there.

Press <F1> for general help. The most important keyboard commands <v> and <V> are used for viewing. If the item is an image, <V> computes a suitable scaling for the color bar, <v> employs the previous setting of the VINCI image buffer to which the item is loaded. This makes it possible to compare series of images by intensity.

The <i> feature displays context related help for the item under the bar. For most items, the <v> and <V> commands are supported. For all items referring to files, <Del> is supported to delete them. A few items also support the <w> command, either to write them to file or to edit them.

3.1 What to Check

Users should familiarize themselves with each of the following items. They are best viewed after a successful pass of step 5, just in order to know that they exist and how they look under normal conditions. They can provide valuable assistance in case of trouble, as explained in the following paragraphs.

3.1.1 Frame End Times (step 0)

shows the times that were found in the image subheaders of the ECAT7 input file. It will upset evaluation completely if the input file does not contain the right times information. You can then stop before step 1 and edit them using the <w> command (confusingly, the editor expects seconds while the <v> command displays minutes). You are then also responsible for manually adapting the “Early Frames”- and “Skip” selectors of the Main Window.

3.1.2 Normalization Params (step 2)

is a file containing the mapping parameters obtained during affine normalization of the Flow Image to the Normalization Template. The parameters are displayed in the text section when <v> is pressed. Together with the “Coregistration Params” (below) they define transformations leading from raw input frames to Normalized Frames. If you want affine Normalization to be recomputed, delete this file. Delete all Normalized frames as well to ensure their recomputation. You can leave the Coregistration Parameters intact if they are OK, in order to save some time during step 3.

The parameters have the following meaning:

rx,ry,rz Euler angles of rotations around the x,y,z axis (in radians)

tx,ty,tz Translation offsets (in mm)

sx,sy,sz Stretch factors (they can be used to compute the brain volume)

kx,ky,kz define the principal axes to which the stretch factors correspond

i.sc. intensity scaling

inverse if set to 1, then the above data refer to the inverse transformation

Press <v>. The **brain volume** computed from sx, sy, sz and the volume of the full brain mask is shown under the normalization parameters.

3.1.3 Coregistration Params (step 3)

are also textfiles, containing the mapping parameters of the coregistrations. They are displayed in the text section when <v> is pressed. Since we use rigid-body-coregistrations, only the first 6 parameters rx,ry,rz and tx,ty,tz are nontrivial. The early frames are not coregistered, hence all entries of their parameter files are trivial.

The purpose of the files is to save coregistration time on every pass through step 3. If the files are found, their parameters will be used for computation of any missing Normalized Frames. If not, coregistrations will be performed and new files written containing the mapping parameters. To repeat coregistration of a frame, delete its Coregistration Params together with its Normalized Frame and rerun step 3.

Their other purpose is to check for patient movements during scantime. Such movements lead to visible changes of the parameters from frame to frame.

3.1.4 Normalized Frame (step 3)

The Normalized Frames should be checked using the <v> command. Alternating it with a double hit of the up-arrow or down-arrow key will show them in succession in the same VINCI image buffer. Since the same color settings are used for all, it is easy to spot frames of outlying intensity, as well as of bad coregistration. Both are possible explanations in case of failure of kinetic analysis. Delete any bad files, repeat step 3 and check again. In case of bad coregistration it may be necessary to also delete their Coregistration Params.

3.1.5 Normalized Frames (step 3)

This item provides a <v> command for simultaneous loading of several normalized frames to VINCI. The color scheme will be borrowed from the VINCI image buffer used for display of single normalized frames or the Sum Image (see below), it is therefore recommended to first adjust this color scheme. The <w> command writes out all Normalized Frames as interfiles.

3.1.6 Sum Image (step 3)

is the decay weighted sum of the Normalized Frames. This is the image that would result if everything was scanned into a single frame and the patient would not move. Therefore, it has the best resolution of all images, providing such anatomical details as cortex structures and the interhemispheral gap. It is the image that should be used to check success of normalization, by fusing it with the atlas or the normalization template. The crispness of the image is an indicator of success of interframe coregistration (correction for patient movement).

3.1.7 Masked Filtered Frames (step 4)

are generated anew upon every pass through step 4. If the Results List is invoked earlier, they are either nonexistent or stem from earlier passes through step 4. Voxel based activity curves are sampled from these frames, hence they are the immediate precursors of the parametric images, and the first thing to check if anything goes wrong.

3.1.8 Border Correction (step 4)

will show an image of the border correction factors that were used in the latest pass through step 4. They have no influence on k_2 and k_3 images, but may be to blame for any funny effects on q_1 images. For 'margin' values of 1 and above little border correction is needed and the image looks flat, while for 'margin'=0 the zones are clearly visible. The correction factors are recomputed upon every pass through step 4.

3.1.9 Zone Image (step 4)

shows an image of the dissection of the brainmask in intensity zones that was used in the latest pass through step 4. The dissection is recomputed during every pass through step 4.

3.1.10 Zoning Layout (step 4)

shows a table of the brainmask distribution to the intensity zones. The zoning plays a role in computing the "Masked Filtered Frames", hence it is one item to check upon abnormalities of the latter. With default settings, there should be 10 to 15 zones, with the largest zone covering some 20 or 30 percent of the brain.

3.1.11 Reference Mask (step 4)

An image of the final reference mask is provided during step 4a in one of VINCI's image buffers. <v> tells which buffer this is and gives some information on the number of voxels of the precursor, before and after padding, and the final mask.

3.1.12 Reference Curve (step 4)

These are text files containing the values of a reference curve. They are another item to check in case you are getting bad parametric images. Every pass through step 4 generates a new file. The files contain header information about the settings that were used to generate them, and let you know if they apply to the current settings or not. Thus you can reckognize the current version. Use <Del> to delete any files.

4 Files and File Formats

Resource files distributed with this release are in subdirectory templates/mp4a2. They include all files distributed with the previous version, and some new reference mask precursor files discussed below.

4.1 VINCI Image Volumes

The voxels are stored as linear arrays of 4-byte floats, called volumes. Each volume is a sequence of planes (i.e. voxels having the same z coordinate). Each plane is a sequence of columns of voxels sharing the same y coordinate. In short, 3 dimensions are mapped canonically onto a linear array such that x moves first, then y, then z.

4.2 Input Files

MP4Aml expects an ECAT7 multiframe format of arbitrary size. Each frame should have a time entry (end time of frame after tracer injection).

4.3 Normalization Template and related Files

The distribution template **PET1.v** is an ECAT7 format of 91×109×91 voxels sized 2×2×2 mm. It defines what we call **Evaluation Format**, it is used exclusively during steps 4 and 5 and becomes the format of the output images. All other image resources provided with this release are Interfile images in Evaluation Format: The brain mask

fullbrainmask1.img, the atlases **atlas1.img** and **atlas2.img** and the reference mask precursors.

4.4 Reference Mask Precursor Files

putamen1.txt and **cerebellum1.txt** are textfiles containing lists of numbers, which are zero based linear addresses into a VINCI image volume in Evaluation Format. With this release, we provide the same masks as interfile images named **putamen1.img** and **cerebellum1.img**. They match the putamen and cerebellum masks contained in atlas1.img, whose sizes are 1557 and 23229 voxels, respectively. In addition, we provide unilateral left and right putamen and cerebellum mask precursors named l_putamen1.img, r_putamen1.img, l_cerebellum1.img and r_cerebellum1.img. They have been used for the L,R-Comparisons of [1], chapter 14. Note that the unilateral cerebellum masks require negative padding (see section 5.4) in order to keep them from overlapping.

Users can of course create their own mask precursors as interfiles. Their format must match the format of PET1.v.

4.5 Migrating to a different Template

Note that "Evaluation Format" (section 4.3) is defined by PET1.v, which currently has $91 \times 109 \times 91$ voxels sized $2 \times 2 \times 2$ mm. If PET1.v was to be replaced by a different template in the future, that format might change and render invalid all current resource files, namely: atlas1.img, atlas2.img, fullbrainmask1.img and the precursor files of section 4.4. Users would have to supply an atlas, a full brain mask and a reference precursor file of their own. All would have to be in interfile format and matching the volume and pixel sizes of the new template.

The current atlas1.img has been downloaded from the MNI website and resliced to Evaluation Format. atlas2.img has been generated by the author by dissecting every region of atlas1.img along the interhemispheral border, given by the equation x=45.5 (where x is the, 1-based, first VINCI image voxel coordinate). In addition, new numeric labels have been defined for all regions, which are odd in the hemisphere of $x \leq 45$ and even in $x \geq 46$. They are documented in regions2.txt of section 2.2.

4.6 Output Files

4.6.1 Naming

Filenames are generated using the following building blocks:

- **Dots** are used as separators, dividing the generated name in 3 or 4 sections.
- **PAT** denotes a copy of the "patient identifier" by which we understand the name of the dynamic PET input file minus its extension .v.
- **FF** denotes the frame number to which a file refers.
- **XX** denotes a "multiplexing number" generated to distinguish a filename from previously generated filenames, thus ensuring the files do not overwrite each other. That number is 1 for the first generated file of a series. Otherwise, it is 1 plus the highest multiplexing number found in an existing sibling file.

4.6.2 Parametric Image Files

are automatically written to the patient directory when the auto-save-all checkbox is checked during a pass through step 5. They are interfiles named **PAT.mm.XX.img** where *mm* is a modality descriptor matching the description in the compute-what groupbox, such as *k3*, *k3dev* or *simcr*, and *img* is the filename extension for interfiles. The associated header file, PAT.mm.XX.hdr, is a text file containing standard interfile descriptors, along with extra information on the settings that have been used for generating the parametric image. Filename example: M01002.k3.17.img with M01002.k3.17.hdr.

4.6.3 Normalized Frames

are exported as interfiles to the patient directory when the user hits the <w> key on the "normalized frames" item of the Results List, see section 3.1.5. This feature serves as a bridge connecting MP4Aml's internal file format to the external world. Filenames are **PATnFF.img** / hdr, meaning that the letter n, the frame number and the extensions .img and .hdr are appended to the patient name. Examples: M01002n9.img, M01002n10.img.

4.6.4 Masked Filtered Frames

as generated by step 4b, are exported as interfiles to the patient directory when the user hits the <w> key on the "Masked filtered frames" item of the Results List. This feature serves as a bridge connecting MP4Aml's internal compression format to the external world. Filenames are **PATmFF.img** / hdr, meaning that the letter m, the frame number and the extensions .img and .hdr are appended to the patient name. Examples: M01002m9.img, M01002m10.img.

4.6.5 Cr Files

containing a reference curve apiece, are automatically written to the patient directory upon every pass through step 4. They are text files containing a header section for documenting the settings and the mask precursor filename that were used for generating the reference mask. Filenames are **PAT_CrXX.txt**, meaning that the patient name is followed by the fixed sequence _Cr and a multiplexing number and the extension .txt.

4.6.6 Regional Files

are generated by the user clicking the <Regional> button and following through the procedures of section 2.1. They are text files intended for manual processing by the user, or copy/pasting their contents to other software. Entries of every line are separated by tabs. The files contain a header section for documenting the settings with which the images were generated. The header also contains a copy of the reference curve. The files are written to the patient directory, using **PAT.regional.XX.txt** as filename scheme. Filename example: M01002.regional.1.txt.

4.7 Internal Files

are for internal use by MP4Aml, they cannot be read by other software.

4.7.1 Normalized Frames

are written to the patient directory during every pass through step 3. Their naming scheme is PAT_nFF.img and PAT_nFF.hdr, which is the patient name followed by _n and the frame number and the extension .img and .hdr. From their counterparts of section 4.6.3 they are distinguished only by the underscore. They are not interfiles themselves, because they have a different header format. Their header files contain the parameters of rigid body coregistration that were determined by the VINCI MMM tool during step 3. The purpose of these files is explained in sections 3.1.4 and 3.1.3. Use the Results List for conveniently deleting them in case you want them recomputed during the next pass through step 3. Section 4.6.3 explains how to export them as interfiles.

4.7.2 Masked Filtered Frames

are written to the patient directory during every pass through step 4. Their naming scheme is **PAT_mf_FF.xpt** and **PAT_mf_FF.hdr**, which is the patient name followed by _mf_ and the frame number and the extension .xpt and .hdr. While the .xpt file contains the volume in a compressed format (storing brain voxels only), the .hdr file contains the settings that were used for generating the associated zoning masks (see [1], section 10.4.3) which determine the order in which the voxels are stored.

The purpose of these files is explained in section 3.1.7. They are recomputed during every pass through step 4, and can be conveniently deleted using the Results List. Section 4.6.4 explains how to export them as interfiles.

5 Settings Reference

For the basics see sections 1.5 and 1.6. For completeness, here is a list of all settings sorted by location of their edit fields. We label the settings by the following classification markers:

- **(d)** "default guarded"

  These settings are under automatic control in order to help users avoid unvoluntary modifications. User modifications are remembered between the sessions in order to facilitate their application to a large sample of patients, if intended. However, every deviation from the default produces a Startup Message (section 1.6). In addition, deviations are reported by the <Report> button and restored to their defaults by the <Native> button of the <Settings> menue.

- **(u)** “user”

  There are no defaults for this type of setting, hence there is no automatic control. For convenience, user preferences are memorized between the sessions.

- **(p)** “permanent”

  These settings can be changed during a session, but they are restored to their defaults in every new session.

- **(A)** “automatic”

  These settings depend on the framing of the input file. They are computed during step 0 and can be edited by the user only at the start of step 1. Such modifications are not remembered in the next session.

5.1 Main Widget Settings

are edited on the main widget of the MP4A tool:

Early frames (A)

is the number of “early frames” used for computing the “Flow Image” (see section 1.7, step 1). Unlike the remaining frames, they are not corrected for patient movement. During step 0, the number of early frames is set to cover the first 10 minutes of scantime. If you want a different number, stop before step 1 and apply it manually. This must be repeated for every patient. In-depth information on the role of the early frames is given in [1], section 9.2.1.

Gaussian FWHM (d)

default: 8mm. FWHM is the width of the Gauss filter applied to every frame during image preprocessing (see section 1.7, step 4b, and [1], sections 2.9 and 9.3). The default of 8mm is the result of validation efforts documented in [1], section 10.6.

NLS checkbox (d)

default: on. When checked, kinetic analysis is performed by Nonlinear Least Squares fitting, otherwise, by the COLOGNE method. See section 1.5.

Skip selector (A)

indicates the number of leading frames to be excluded from kinetic analysis. During step 0, this number is set to cover the frames of the first 60 seconds of scantime. If you want a different number, stop before step 1 and apply it manually. This must be repeated for every patient.

In-depth information on why it has proven favorable to exclude the frames of the first minute is provided in [1], section 12.1.

Modify Button (u)

brings up a panel of checkboxes for selecting the image modalities to be computed. Every checked box produces one parametric image during step 5. You will probably want k_3 checked to compute a parametric k_3 image. Most of the other modalities are explained in [1], and can be found through its glossary. Exceptions are ‘ces’ which is obsolete, ‘dwsum’ which is the decay weighted sum of all masked filtered frames, ‘corr’ which is only available for COLOGNE runs and gives the maximum correlation found between the left and right hand sides of equation (3.13) of [1].

After making your selection, click the panel’s close field, and the display of the “Compute what” group will be actualized.

auto-save-all checkbox (u)

If this box is checked, each parametric image computed during step 5 is automatically saved to the patient directory. For details on these files see section 4.6.2.

5.2 Settings Menue, General

Memory usage (d)

default: 104857600 bytes=100 megabytes. Use this field to limit the amount of memory used for simultaneous storage of image frames during the computations of step 4 and 5, if that turns out to be necessary. Images will then be processed in slices, which may lead to increased computation time.

Show experimental features (p)

default: off. Checking this box will bring back some experimental features of the previous release as items (mostly buttons) of the main widget. While they are still functional for performing calculations, Monte Carlo simulations or viewing activity curves, there exists no documentation of these features and they are not always stable.

Pandora (d)

default: off. This enables some other experimental properties, such as supplying external Cr files which were needed for the computations of [1], chapter 14. In addition, it allows step 4 to run faster by using existing copies of masked filtered frames, that may have been generated in previous sessions. Use of this feature is strongly discouraged since it entails considerable risk of corrupting your data.

5.3 Settings Menue, Steps 2 and 3

The **XML script for affine normalization** (d) is available for editing after clicking on its button on the Step 2 panel. It controls VINCI's MMM tool when computing the 12 parameters of the affine mapping by which the Flow Image is mapped to the Normalization Template (see section 1.7). To regain its default, click its button to open the editor, there click <Show Standard> and then <OK>. The editor should close, and the Settings Report (of section 1.6) should report no more differences of this item. Changes to the XML script can be made in compliance with the properties of the MMM tool, see its documentation.

The **XML script for coregistration** (d) is available for editing after clicking on its button on the Step 3 panel. It controls VINCI's MMM tool which computes the 6 parameters of the Rigid Body coregistration of every "late frame" to the Flow Image (see section 1.7). It is handled like the other XML script, see above.

5.4 Settings Menue, Step 4

Number of voxels (d)

default: 1557. This is the final size of the reference mask generated during step 4a. Section 10.3 of [1] explains in detail how that mask is generated, referring to this number as '**regionsize**'.

Padding thickness (d)

default: 3. This number governs growing (or shrinking, if negative) of the precursor mask, which is applied during generation of the final mask in step 4b. It corresponds to the parameter '**padding**' of [1], section 10.3, where this procedure is explained.

Preselect ratio (d)

default: 1.0. Currently, voxel preselection is switched off completely for reasons explained in section 10.4 of [1]. Any value below 1 would lead to preselection based on *simcr*, such as to keep, for instance, 65 percent of all voxels if it were set to 0.65. However this feature has not been tested recently and should therefore not be used.

Zoning quotient (d)

default: 0.85. Quotient between thresholds used for intensity zoning. Referred to as '**qzoning**' in section 1.7, step4b and in [1], section 10.4.3.

FWHM3 (d)

default: 8 mm. Gauss filter width applied during generation of the zones during step 4b in order to achieve compactification. See [1], section 10.4.3.

margin (d)

default: 1. 'margin' controls the application of the zoning masks during computation of the masked filtered frames. For explanation, see [1], section 10.4.3. The default choice of 1 results from validation efforts documented in [1], section 10.6.

5.5 Settings Menue, Step 5

General Section

Add, Level, Remove

A still functional, undocumented feature used to process multiple parameter sets as in [1], appendix A.3.

Interpolation Method

Obsolete in this version and therefore deactivated. Interpolation is always by a cubic spline.

Use k_{3r}-correction (d)

default: off. An optional feature for correction of reference curves sampled from nonideal reference regions. Summarized in [1], section 16.4, with theoretic background given in [1], section 3.2.3.

k_{2r}, k_{3r} (d)

default: 0.1, 4.0. These are the parameters used for k_{3r}-correction. The defaults apply to putamen, not considering the possibility of partial volume effects. For cerebellum as reference, k_{3r} should be lowered to about 1.5. See [1], section 15.7.

COLOGNE specific

Kinetic analysis by the COLOGNE method ([1], section 3.3) is performed using weighted linear regression and weighted correlations. The most favorable weighting turned out to be the product of C_r proportional weights ([1], section 7.3) and Decay Weights ([1], section 2.7, validation in section 7.3).

Use C_r weighting (d)

default: on.

Use Decay weighting (d)

default: on.

NLS specific

NLS fitting minimizes the sum of weighted squares as in equation (4.2) of [1]. Validation ([1], section 7.3) revealed Decay Weighting as the optimal strategy.

Use C_r weighting (d)

default: off

Use Decay weighting (d)

default: on

Max. iterations (d)

default: 20. Corresponds to n_1 as of [1], section 5.3.

breakoff step lengths

underway (d)

default: 1e-7. Corresponds to l_1 as of [1], section 5.3.

final (d)

default: 0.0001. Corresponds to l_2 as of [1], section 5.3.

Starter points (d)

defaults: (1;0.1;0.1) and (-1;0.8;0.3). Correspond to S_1 of [1], section 5.2, and S_4 of [1], section 5.6. S_4 is disabled as long as its first component is negative. To enable it, replace -1 with 1. NLS will then perform competing runs from both starting points and pick whichever solution has the smaller squares. As reported in [1], section 5.6, this reduces the number of suboptimal solutions.

lambda (d)

default: 0.9. If positive, lambda switches on step size control as in [1], section 5.4. Its adoption improved the convergence properties of NLS.

5.6 Settings Menue, Regional

Atlas (d)

default: atlas2.img. Discussed in section 2.2.

Region Descriptor (d)

default: regions2.txt. Discussed in section 2.2.

Intensity File (d)

default: edit field is empty. Discussed in section 2.3.

Mark failed voxels with (d)

default: 0. Discussed in section 1.8.1.

Recognize as failmark (d)

default: 0. Discussed in section 1.8.1.

k_3 confinement

apply (d)

default: off. Discussed in section 1.8.2.

upper border (d)

default: 1. Discussed in section 1.8.2.

lower border (d)

default: 1e-6. Discussed in section 1.8.2.

treat violation as failure (d)

default: on. Discussed in section 1.8.2.

References

[1] Hohmann C, PhD thesis, "*Kinetic Analysis of dynamic MP4A PET Scans of Human Brain using Voxel based Nonlinear Least Squares Fitting*", University of Cologne, 2009