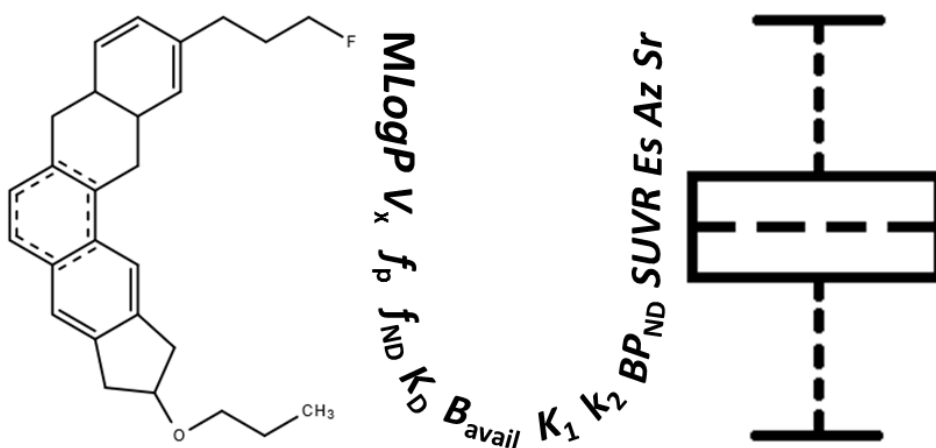


Radiotracer Screening with



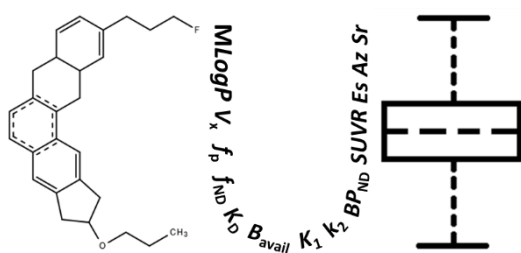
RSwCUI

Manual Version 1.0

Updated on 1st November 2017

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INTRODUCTION

To facilitate the development of new radiotracers, the methodology proposed (refer to reference) was developed into a program. The methodology encompassed both physicochemical and pharmacological parameters of radiotracers to simulate possible in vivo kinetics of radiotracers at variable conditions. The use of biomathematical modelling and simulation would complement high throughput screening by allowing simultaneous and rapid evaluation of many candidate radiotracers. Evaluating the possible diagnostic capability of radiotracers via clinical usefulness index (*CUI*) would support decision making in moving candidate radiotracers to clinical diagnostic application. The following methodology can be extended to other radiotracers showing similar kinetics as amyloid radiotracers.

SYSTEM REQUIREMENTS

Matlab is required to run RSwCUI: <http://www.mathworks.com/products/matlab/>.

The program is written and tested using Matlab R2014b. The program should be workable on both Windows and Linux environment.

Lipophilicity (*MLogP*) and McGowan Volume (V_x) are determined using commercial software, dproperties (Talete, Italy): http://www.talete.mi.it/products/dproperties_description.htm

Note: The use of other similar software to determine both lipophilicity and molecular volume is possible but results will be different and may not correlate well with author's results.

ABBREVIATIONS

<i>CUI</i>	Clinical Usefulness Index	TAC	Time Activity Curve
<i>SUVR</i>	Standardized Uptake Values Ratio	K_1	Influx rate constant
<i>LogP</i>	Logarithmic partition coefficient	k_2	Efflux rate constant
<i>MLogP</i>	Moriguchi <i>LogP</i>	BP_{ND}	Non-displaceable binding potential
V_x	McGowan Volume (Molecular Volume)	HC	Healthy Control
f_p	Free fraction in plasma	MCI	Mild Cognitive Impaired
f_{ND}	Free fraction in tissue	AD	Alzheimer's Disease
B_{avail}	Available target binding sites	ROC	Receiver Operating Characteristics
K_D	Dissociation Constant	A_z	Area under ROC curve
GUI	Graphical User Interface	E_s	Effect size
TCM	Tissue Compartmental Model	S_r	<i>SUVR</i> ratio

OVERVIEW OF PROGRAM

An amyloid biomathematical model (Figure 1A) was developed to predict *SUVR* values in subject conditions of HC, MCI and AD using key pharmacological and physicochemical parameters of a radiotracer. To encompass subjects with variable load conditions, the model was applied together with population realizations to simulate 1000 time activity curves (TACs) and *SUVR* values for each subject condition of HC, MCI and AD (Figure 1B). The *SUVR* values generated were then used to determine *Az*, *Es* and *Sr* for each subject-conditions pair of HC-MCI and MCI-AD. The resulting *Az*, *Es* and *Sr* were averaged and their product determined the *CUI* values for each amyloid radiotracer (Figure 1B). For details, please refer to the references.

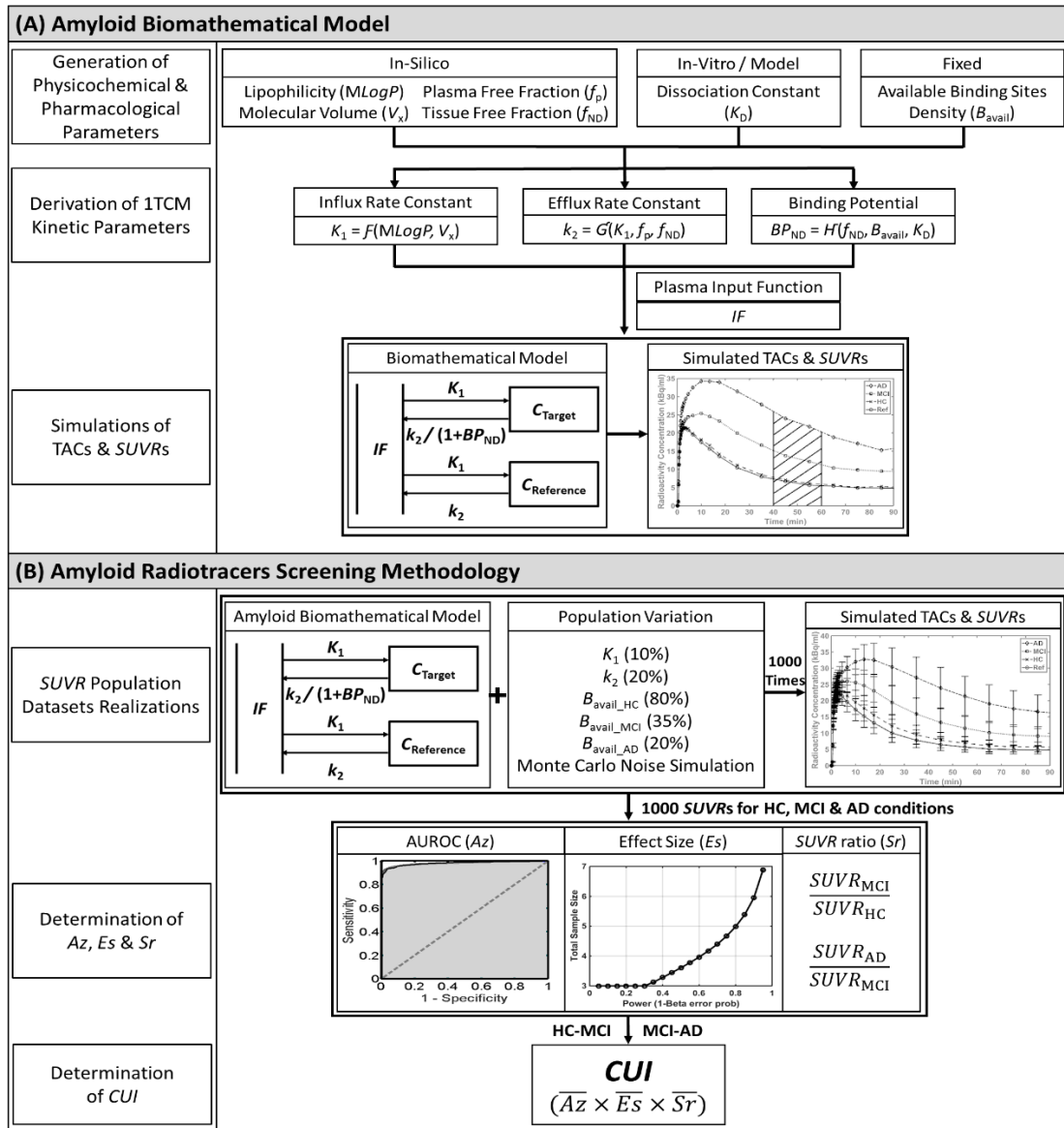


Figure 1: Overview of screening process of amyloid PET radiotracers, including (A) Amyloid biomathematical Model and (B) Amyloid Radiotracers Screening Methodology.

FEATURES OF PROGRAM

Figure 2 below shows the 8 main features of RSwCUI graphical user interface (GUI) in Matlab, as highlighted in various colours.

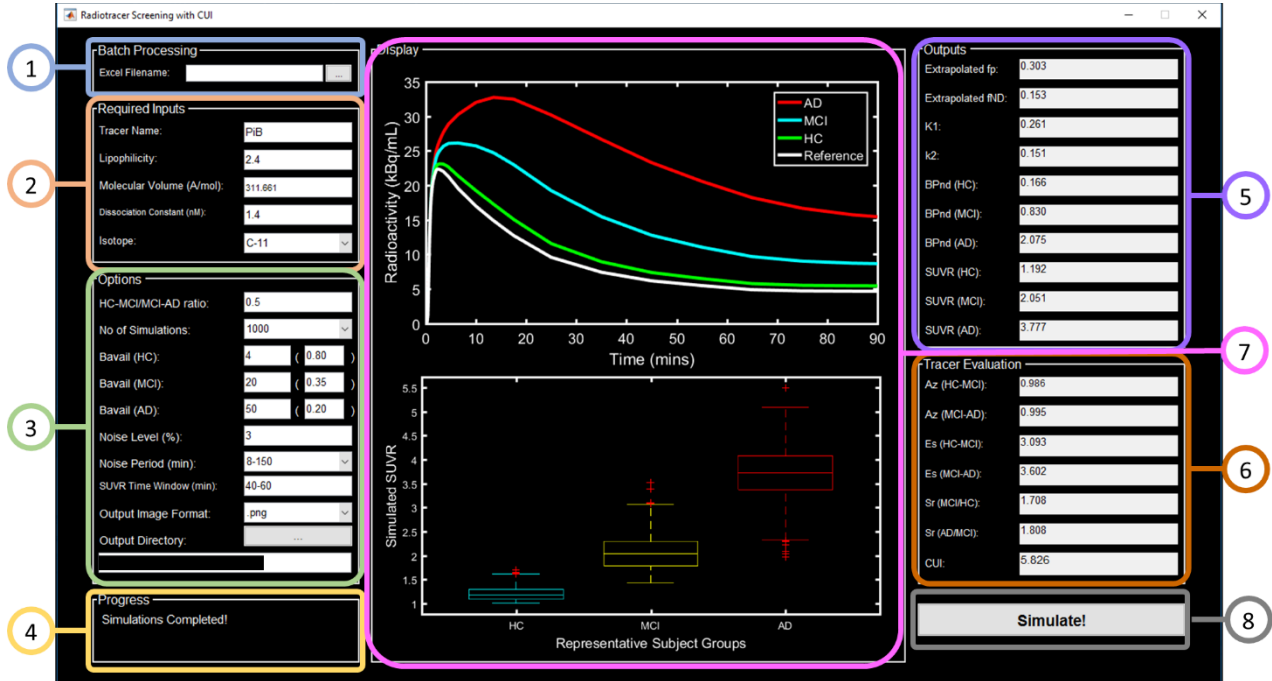


Figure 2: Overview of RSwCUI graphical user-interface in Matlab.

1. Batch Processing

For evaluating many tracers, batch processing is recommended. The required input data listed in “Inputs Panel” (Refer to next section) are filled into an excel file in the same format as the template provided. All the tracers listed will be evaluated. If any data in the template is missing or in the wrong format, the program will terminate.

2. Required Inputs

5 inputs are required for simulations. If batch processing file is selected, the inputs will be filled automatically from the excel file selected. If only a single radiotracer is evaluated at any one time or batch processing input is empty, simulations will only start if all areas in the input panel are filled. The details of each input are described below:

Tracer Name

Name of the radiotracer for evaluation.

Lipophilicity, $LogP$

Lipophilicity of a compound can be expressed in terms of the logarithmic partition coefficient of the radiotracer in water and octanol at equilibrium or $LogP$ in short. Either in-silico (computer simulated) or measured $LogP$ of the radiotracer can be used. Many in-silico $LogP$ are available for evaluation. However, the author recommends the use of Moriguchi $LogP$ ($MLogP$), which resulted in outputs with the best correlation with literature-cited K_1 , k_2 , BP_{ND} and $SUVR$ values.

Molecular Volume, V_x

McGowan volume (V_x) is used to represent the molecular volume of the radiotracer. The unit of V_x as determined from dproperties (Talete, Italy) is Å/mol and will be converted automatically to cm³/mol/100 in the program.

Dissociation Constant, K_D

Dissociation constant (nM) is the only in-vitro parameter required in tracer evaluation and is measured from binding studies using either synthetic A β fibrils or AD brain homogenates. It is then determined from Scatchard plot. As K_D is the most sensitive parameter in the model, the author recommends that the same protocol is used in measuring K_D . The use of a reference radiotracer that is clinically applied (e.g. [¹¹C]PIB) is highly recommended for more accurate radiotracer evaluation.

Isotope

The amount of noise in clinical PET data is dependent on the isotope, in which shorter half-lives led to reduced counts from radioactive decay and hence resulted in greater amount of noise in in-vivo measurements. The current program only encompassed 2 types of radioisotopes – ¹¹C and ¹⁸F.

3. Options

10 options are available for modifications. The default settings were used by the authors in their model development and hence had been tested. The default options settings are recommended.

HC-MCI / MCI-AD ratio

HC-MCI / MCI-AD ratio refers to the ratio of HC-MCI and MCI-AD subject-conditions pairs for the determination of *CUI* values. For the development of radiotracers that show greater *SUVR* difference at lower target densities, a larger HC-MCI / MCI-AD ratio should be input and vice-versa. The default setting is 0.5, indicating the weightage for both HC-MCI and MCI-AD subject-conditions pairs are equal.

No of Simulations

The number of simulations refers to the number of *SUVR* values simulated with population variation for target regions of subject conditions of HC, MCI and AD each and the reference region. 3 values can be selected: 1000 (default), 2000 and 3000. The greater the number, the lesser the amount of variations in outcome *CUI* values but the longer the time required to evaluate each tracer.

B_{avail} (HC, MCI, AD)

B_{avail} (HC, MCI, AD) options refer to the available target binding densities, (e.g. in the case of amyloid radiotracers, A β binding sites), for subject-conditions of HC, MCI and AD respectively. The first box refers to the representative fixed value followed by the amount of variations in human population in the brackets. The values used will affect the *SUVR* population distributions of subject conditions of HC, MCI and AD and hence outcome *CUI* values. The default fixed B_{avail} values (HC, MCI, AD) are 4, 20 and 50 nM while the fractional population variations are set to 0.8, 0.35 and 0.20 respectively. These values had been optimized for the evaluation of amyloid radiotracers using the default input functions and other settings. For other types of radiotracers (e.g. Tau), adjustments may be required to simulate *SUVR* population variations similar to that of clinical results. However, the same settings should be used to evaluate the same type of tracers.

Noise Level

Noise level (in %) refers to the amount of noise in PET clinical measurement. Noise was added to simulate time activity curves (TACs) with population variations. The default setting of 3% noise level was applied within the time period (refer to Noise Period) specified.

Noise Period

Noise period (min) refers to the time period within the simulated time activity curve where noise is applied. The default time period is 8 to 150 min and is written in the following format “8-150”.

3 options are available: 8-90, 8-120 and 8-150.

SUVR Time Window

SUVR time window refers to the time window where *SUVR* is determined. The default time window for determining *SUVR* is 40 to 60 min and is written in the following format of 40-60.

Output Image Format

For each radiotracer evaluation, 2 plots will be generated – (1) TACs simulated for target regions of subject conditions of HC, MCI and AD each and the reference region, and (2) *SUVR* population distributions across subject conditions of HC, MCI and AD. For batch processing, an additional plot will be generated showing the CUI distribution across the various radiotracer evaluated. These plots will be automatically saved in “.mat” format. If “.png” is selected, an additional plot will be saved in the selected output directory.

Output Directory

The output directory refers to the folder where the plots and output data will be saved to. By default, the program directory is selected.

4. Progress Information Panel

Progress information panel will display the progress of the simulation. For batch processing, the name of the tracer being evaluated will be displayed. Error messages are also displayed here.

5. Simulations Outputs

10 outputs will be generated for each radiotracer.

Extrapolated f_p and f_{ND}

Free fraction in plasma (f_p) and free fraction in tissue (f_{ND}) are extrapolated from the model stated in the reference journal, using *MLogP*. The values will be different if other lipophilicity values are used instead of *MLogP*.

1 Tissue Compartmental Model (TCM) Kinetic Parameters

1 Tissue Compartmental Model (1TCM) kinetic parameters were derived from radiotracer's in-silico and in-vitro physicochemical and pharmacological parameters. Influx rate constant from the artery to brain tissue (K_1) and efflux rate constant from brain tissue to the vein (k_2) are the same for all subject conditions of HC, MCI and AD. The non-displaceable binding potential of the radiotracer to target molecules (BP_{ND}) are determined, assuming the respective amyloid load (B_{avail}) for subject conditions of HC, MCI and AD are 4, 20 and 50nM.

Standardized Uptake Values Ratio (*SUVR*)

The *SUVR* values stated in the output panel assumed perfect conditions where no noise and fixed B_{avail} conditions of 4, 20 and 50 nM for subject conditions of HC, MCI and AD were applied.

6. Tracer Evaluation Outputs

The area under Receiver Operating Characteristics (ROC) Curve (*AUROC*, *Az*), effect size (*Es*) and *SUVR* ratio (*Sr*) for subject-conditions pairs of HC-MCI and MCI-AD are calculated from the simulated *SUVR* values for subject conditions of HC, MCI and AD. The values of *Az*, *Es* and *Sr* for subject-conditions pairs of HC-MCI and MCI-AD are displayed respectively and are used together with HC-MCI / MCI-AD ratio to determine the final outcome value of *CUI*.

7. Display Panel

For each radiotracer, 2 plots will be displayed:

- (1) Top figure: TACs simulated for target regions of subject conditions of HC, MCI and AD each and the reference region.
- (2) Bottom figure: *SUVR* population distributions across subject conditions of HC, MCI and AD.

At the end of batch processing, the top figure will be replaced with *CUI* distribution plot, while the bottom plot will be removed.

8. Simulate Button

To start the simulation process, click on the "Simulate" button to start simulations of both individual radiotracer evaluation and batch processing. Note that batch processing will override

individual data input. Clear the data in batch processing to run simulations for the individual tracer.

STARTING THE PROGRAM

Run the program “RSwCUI.mat” either from the editor by clicking Matlab’s “run” button, or type “RSwCUI” in the command line.



At initial start-up, if the program folder and sub-folders directories were not added to Matlab’s path, the following directory prompt will appear:

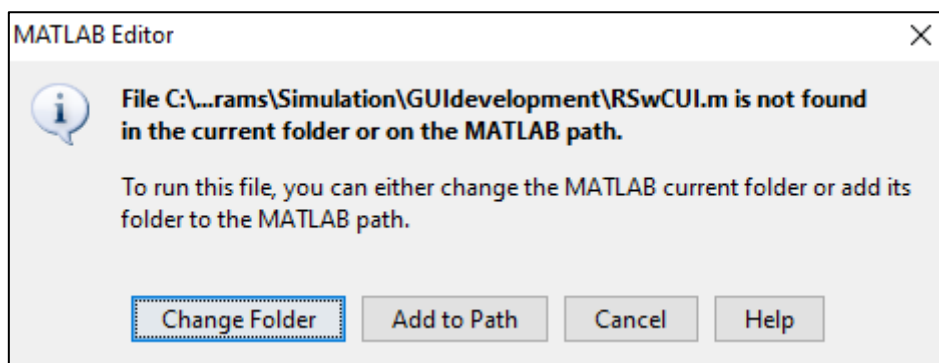


Figure 3: Matlab editor directory prompt at first start-up.

Either “Change Folder” or “Add to Path” can be selected as sub-folders will be added automatically to the path.

If the directory is not set on the subsequent occasion, a directory prompt will appear. Select the main folder where “RSwCUI.mat” file is located.

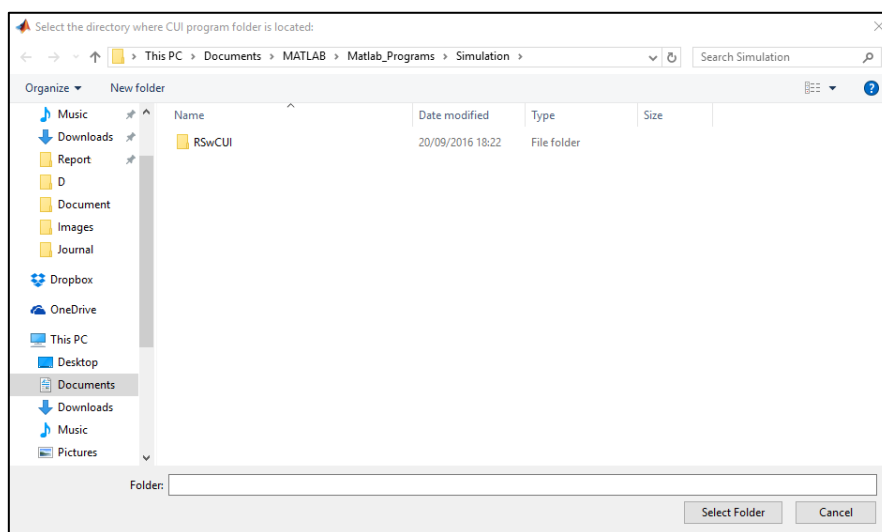


Figure 4: Select directory prompt.

Once all the required paths have been added, the following confirmation statement will appear in the command window of Matlab (Figure 5) and the GUI will appear (Figure 6).

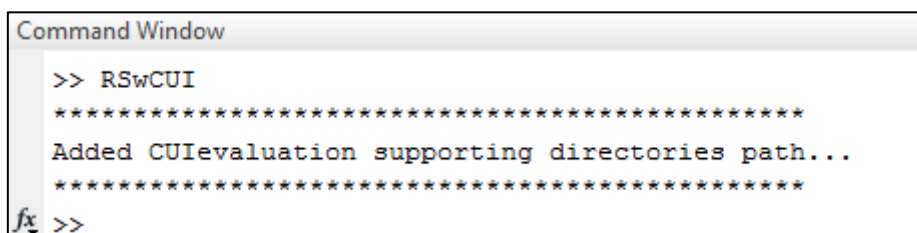


Figure 5: Confirmation statement shown on command window after all required R_{Sw}CUI paths have been added.

Figure 6: Start-up view of R_{Sw}CUI GUI.

RUNNING THE PROGRAM

Filling the Inputs

For evaluating a single radiotracer, input the required fills in the “Required Inputs” section (Refer to “Features of Programs”) as shown in Figure 7.

Figure 7: Required Inputs section of R_{Sw}CUI GUI.

For evaluating multiple radiotracers continuously, carry out batch processing by selecting the appropriate excel file.

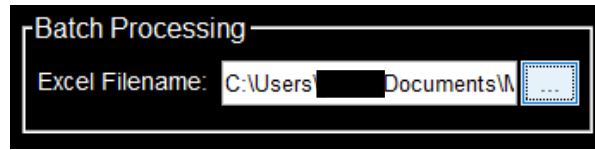


Figure 8: Batch Processing section of R_{Sw}CUI GUI

For ease of referring to the results, especially when batch processing is selected, select the output directory in the “Options” section of R_{Sw}CUI GUI.



Figure 9: Select the Output Directory in Options section of R_{Sw}CUI GUI

After filling in appropriate inputs, click on the “Simulate!” button to start simulations for both individual tracer evaluation and batch processing.

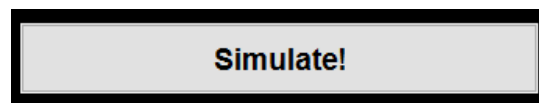


Figure 10: Simulate button of R_{Sw}CUI GUI

Tracking Simulation Progress

Look at the Progress Information Panel (refer to “Features of Programs”) to track the simulation progress. At first start-up of the GUI, the following information will be displayed:

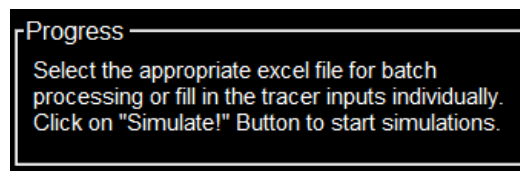


Figure 11: Information displayed at first start-up of R_{Sw}CUI GUI

During tracer evaluation, the following information will be displayed accordingly:

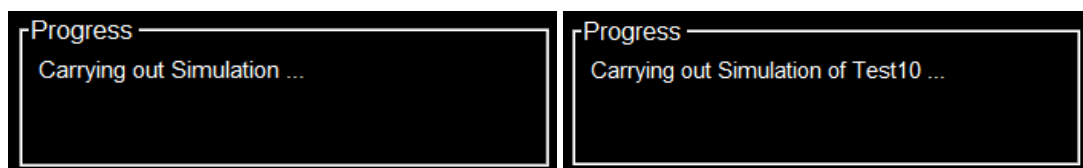


Figure 12: Information displayed during individual tracer evaluation (Left) and batch processing (Right) of R_{Sw}CUI GUI

At the end of tracer evaluation, the following information will be displayed accordingly:



Figure 13: Information displayed at the end of individual tracer evaluation (Left) and batch processing (Right) of R_{Sw}CUI GUI

Displays

During tracer evaluation, the predicted TACs will be shown in the display panel, while 1TCM and *SUVR* parameters will be displayed in the Outputs panel as shown in Figure 14. During population simulations, the display on RSwCUI interface will remain the same (Figure 14) until completion of population simulation and *CUI* determination as shown in Figure 15.

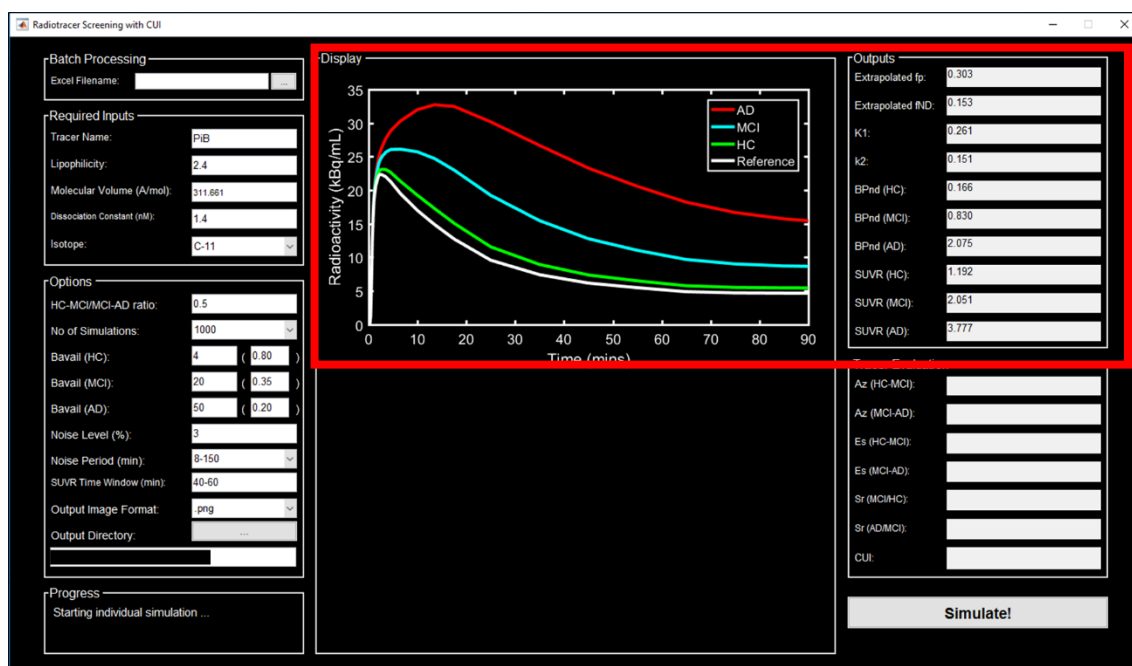


Figure 14: Display view during prediction of basic 1TCM and *SUVR* parameters.

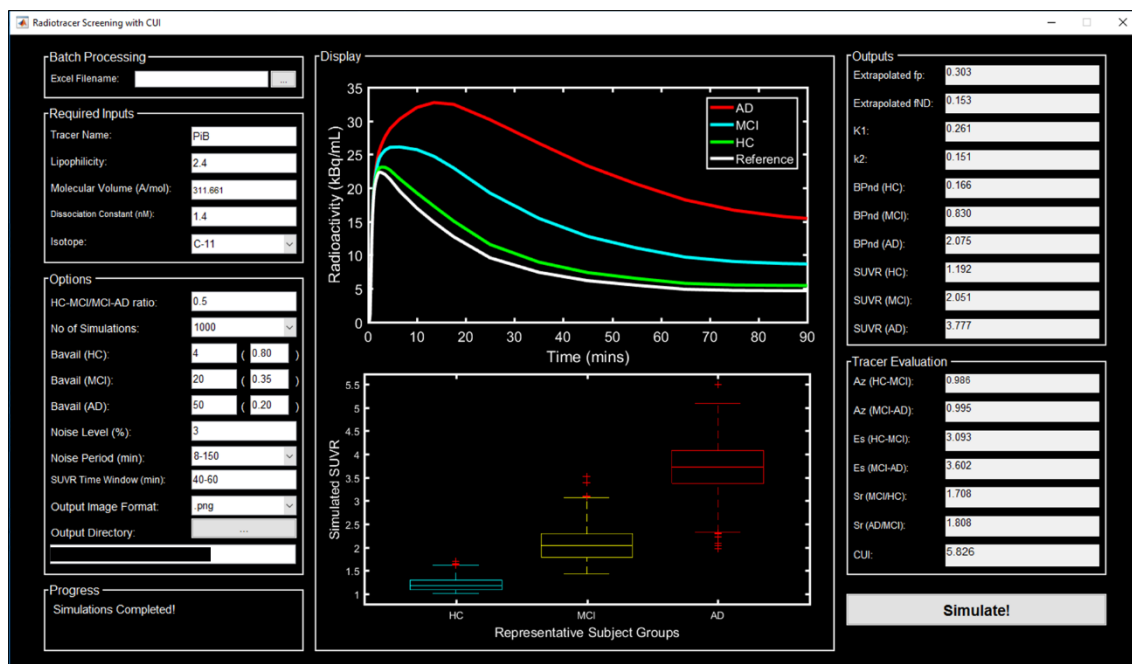


Figure 15: Display view when tracer evaluation is completed with *SUVR* population plot and *CUI* outcome displayed.

For batch processing, the display as shown in Figure 15 disappears quickly to start the evaluation of the next radiotracer. The values displayed in Outputs and Tracer Evaluation panels are determined based on the radiotracer currently being evaluated. At the end of batch processing, *CUI* distribution plot will be shown as shown in Figure 16.



Figure 16: Display view when batch processing is completed with *CUI* distribution plot displayed.

Outputs

For each radiotracer, 2 figures will be generated: (1) TACs simulated for target regions of subject conditions of HC, MCI and AD and the reference region (Figure 17A), and (2) *SUVR* population distributions across subject conditions of HC, MCI and AD (Figure 17B). These figures will be saved automatically in “.mat” format. If “.png” is selected in “Options” panel, the figures will also be saved in “.png” format. The images will be saved in the selected output directory selected.

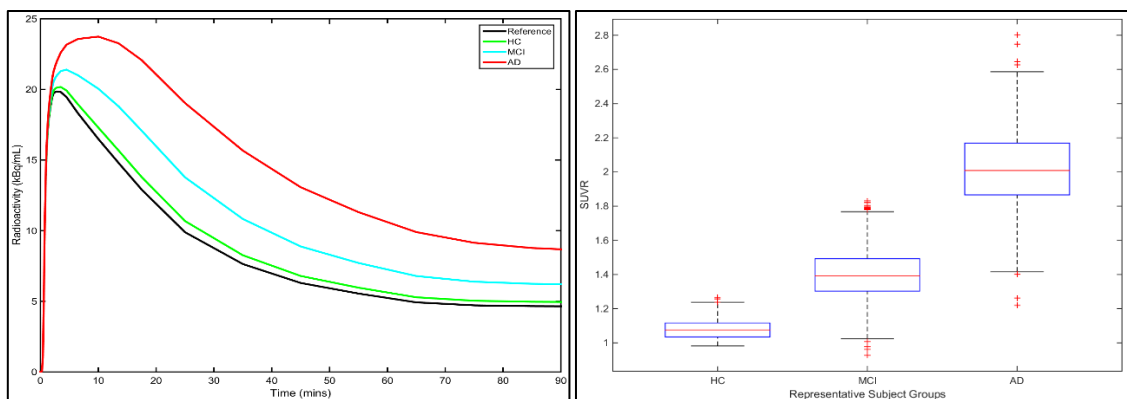


Figure 17: (A) TACs simulated for target regions of subject conditions of HC, MCI and AD and the reference region, (B) *SUVR* population distributions across subject conditions of HC, MCI and AD

For batch processing, *CUI* distribution figure is also saved as shown in Figure 18.

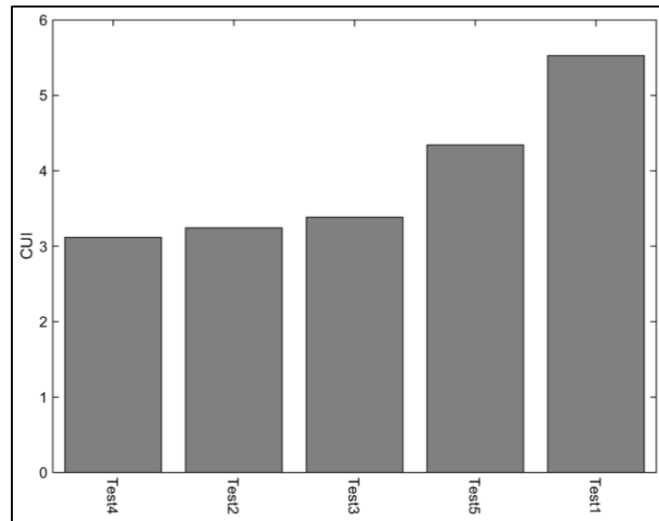


Figure 18: *CUI* distribution plot saved for the all the tracer listed in the excel file for batch processing.

The output of tracer evaluation is also saved in “<Tracer Name>.mat” file with 5 fields:

- (1) inputParams: The inputs ($MLogP$, V_x ($\text{cm}^3/\text{mol}/100$), f_p , f_{ND} , K_D and half-life of radioisotope) required for 1TCM and *SUVR* simulations.
- (2) kmData: Predicted 1TCM (K_1 , k_2 , BP_{ND_HC} , BP_{ND_MCI} , BP_{ND_AD}) and *SUVR* values based on ideal situations.
- (3) populationData: Simulated 1TCM (K_1 , k_2 , BP_{ND_HC} , BP_{ND_MCI} , BP_{ND_AD}) and *SUVR* values with population variations and noise.
- (4) CUIdata: A_z , E_s and S_r for subject-conditions pairs of HC-MCI and MCI-AD and outcome *CUI* value
- (5) Options: All the Options used for simulations and determining *CUI* values.

For batch processing, the predicted 1TCM (K_1 , k_2 , BP_{ND_HC} , BP_{ND_MCI} , BP_{ND_AD}) and *SUVR* values, as well as A_z , E_s and S_r for subject-conditions pairs of HC-MCI and MCI-AD and outcome *CUI* value will be compiled into a single file and save as an excel file “CUIoutput_<date>.xlsx” in the output directory.

TROUBLESHOOT

RswCUI program only checks the inputs and issues in saving output files. The program was written using basic function and hence there should not be any issues in running the program.

However, should there be any incompatibility issues or fall over of the program, please contact the author (refer to reference).

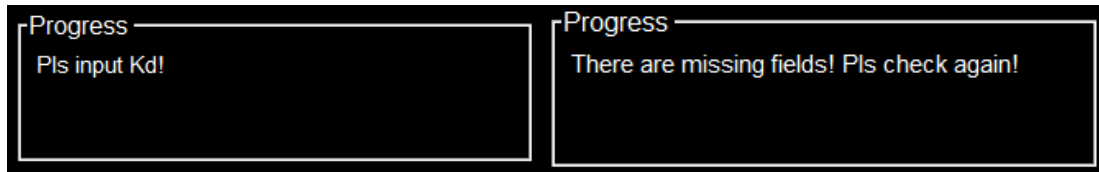


Figure 19: Progress information indicating missing fields for individual tracer evaluation (Left) and for batch processing excel inputs (Right)

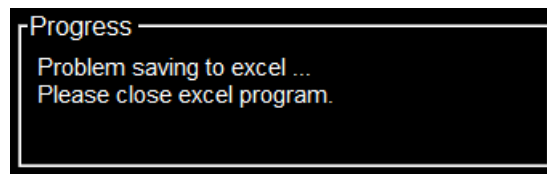


Figure 20: Progress information indicating issues in saving batch processing data into excel file.

TEMPLATE

CUItemplate.xlsx file is provided as a template for batch processing in the sub-folder "Documentation".

REFERENCES

For the use of RSwCUI, please cite the following journal:

1. Arakawa, Y., Nai, Y., Shidahara, M., Furumoto, S., Seki, C., Okamura, N., Tashiro, M., Kudo, Y., Yanai, K., Gonda, K., et al. (2017). Prediction of the Clinical SUV Ratio in Amyloid PET Imaging Using a Biomathematic Modeling Approach Toward the Efficient Development of a Radioligand. *Journal of Nuclear Medicine* 58, 1285–1292.
2. Nai, Y.-H., Shidahara, M., Seki, C., and Watabe, H. (2017). Biomathematical screening of amyloid radiotracers with clinical usefulness index. *Alzheimer's & Dementia: Translational Research & Clinical Interventions* 3, 542–552.

CONTACTS

For future updates and developments, please visit the following website:

<http://www.rim.cyric.tohoku.ac.jp/software/CUI-Software/>

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