**INSTRUCTIONS for cestRARE\_Pagel4**

**For ParaVision 5.1**

**Introduction:**

A copy of these instructions is also stored with the acquisition files, named “cestRARE\_Pagel4\_INSTRUCTIONS”.

This RARE MRI pulse program from Bruker ParaVision 5.1 was modified by Marty Pagel and colleagues (especially Ed Randtke and Kyle Jones) to create a CEST\_RARE MRI acquisition method. Please contact Marty at mdpagel@mdanderson.org or martypagel@gmail.com for questions or suggestions.

This pulse program is provided for free to the MRI research community. Although this pulse program has worked on Bruker MRI scanners with ParaVision 5.1 that have been tested to date,

there is no guarantee that this pulse program will work on all Bruker MRI scanners. Also, while we are collegial researchers, we may not be able to provide assistance due to limited time and other priorities.

This version was developed on July 29, 2018 and is known as the 4th version, cestRARE\_Pagel4.Note that user-edited versions must start with a lowercase letter. Therefore, I have named our method "cestRARE" that starts with lowercase letters.

**Version history:**

Note that previous versions used additional names prior to 2018.

cestRARE\_Pagel4 = written July 2018 at MD Anderson, renamed variables and improved delays,

and improved the respiration trigger code, based on cestFISP\_Pagel4.

**Installation Instructions:**

You need a basic understanding of UNIX / LINUX commands to use these instructions.

There are four general steps (Steps 1-4 below).

1. Move the cestRARE\_Pagel4 directory and files to /home/<your\_login>/src/PV5.1 directory.

If you do not have a "src" sub-directory in your home directory, or a "PV5.1" subdirectory in your src directory, then create one (mkdir ~/src; mkdir ~/src/PV5.1'). Also, create "bin" and "bin/PV5.1" directories in your home directory (/home/<your\_login>/bin/PV5.1). The "bin" directory contains the compiled version of your pulse program, if you edit and recompile programs.

2. Copy files from the cestRARE\_Pagel4 directory to the ParaVision directories. Note that the commands below assume that your ParaVision 5.1 version is stored in a directory named "/opt/PV5.1". Some Bruker scanners store their ParaVision 5.1 version in a directory named "opt/PV51". Therefore, "cd /opt; ls" to determine which directory name your system uses. Then modify the commands below as needed.

cp ~/src/PV5.1/cestRARE\_Pagel4/cestRARE\_Pagel4.so /opt/PV5.1/prog/parx/pub

cp ~/src/PV5.1/cestRARE\_Pagel4/cestRARE\_Pagel4.ppg /opt/PV5.1/exp/stan/nmr/lists/pp

cp ~/src/PV5.1/cestRARE\_Pagel4/cestRARE\_Pagel4.4ch /opt/PV5.1/exp/stan/nmr/lists/pp

cp ~/src/PV5.1/cestRARE\_Pagel4/cestRARE\_Pagel4\_Trigger.mod

/opt/PV5.1/exp/stan/nmr/lists/pp

3. Create a protocol file.

Most Bruker MRI scanners have a directory named

/opt/PV5.1/exp/stan/nmr/parx/routine/S116/<your\_routine\_S116\_directory\_name>

where <your\_login> is your login name. This is the directory where your customized protocols are stored. However, some Bruker MRI scanners use a subdirectory other than "S116" for storing protocols, such as G060. Therefore, you need to check to determine if you have a directory named

/opt/PV5.1/exp/stan/nmr/parx/routine/S116/<your\_routine\_S116\_directory\_name>

or if there is a subdirectory with <your\_routine\_S116\_directory\_name> somewhere else in /opt/PV5.1/exp/stan/nmr/parx/routine/

Then type the following command:

cp ~/src/PV5.1/cestRARE\_Pagel4/cestRARE\_Pagel4

/opt/PV5.1/exp/stan/nmr/parx/routine/S116/<your\_routine\_S116\_directory\_name>

Then edit this file in

/opt/PV5.1/exp/stan/nmr/parx/routine/S116/<your\_routine\_S116\_directory\_name>

I prefer to use the UNIX/LINUX "vi editor". But you can use other editors. For example,

vi /opt/PV5.1/exp/stan/nmr/parx/routine/S116/<your\_routine\_S116\_directory\_name>/cestRARE\_Pagel4

Then change "login" in this file to the target directory in the vi. Type:

:1,$s/<your\_routine\_S116\_directory\_name>/the\_name\_of\_your\_directory/g

Or find the three occurrences of "<your\_routine\_S116\_directory\_name>" in this file and change to your sub directory name in S116.

Then type escape, and type :wq

4. Create files for Image Sequence Analysis

You can view your CEST spectrum in the Image Sequence Analysis tool, in the Image Display & Processing menu, Processing Submenu. Check that you have the following two "isa" directories:

/opt/PV5.1/prog/curdir/<your\_login>/ParaVision/isa/src

/opt/PV5.1/prog/curdir/<your\_login>/ParaVision/isa/obj

then

cp ~/src/PV5.1/cestRARE\_Pagel4/cest\_Pagel\_ISA /opt/PV5.1/prog/curdir/<your\_login>/ParaVision/isa/src/

cp ~/src/PV5.1/cestRARE\_Pagel4/cest\_Pagel\_ISA.so /opt/PV5.1/prog/curdir/<your\_login>/ParaVision/isa/obj/

Note that cest\_Pagel4\_ISA files are the same for all cestXXXX\_Pagel4 pulse programs. Therefore, if you have installed cest\_Pagel4\_ISA files for another cestXXXX\_Pagel4 pulse program, then you have already installed these cest\_Pagel4\_ISA files.

5. Restart ParaVision. If ParaVision is running, stop ParaVision. Then restart ParaVision.

Start a New Patient, New Study, and New Scan. You should be able to select

"cestRARE\_Pagel4" as your new scan protocol.

**cestRARE\_Pagel4 Parameters:**

Edit Method to see all of the parameters.

Measuring Method = cestRARE\_Pagel4

Presaturation Delay = 0.01 ms

This delay is set to 10 microseconds by default, and therefore is negligible. The Presaturation Delay occurs before the loop of saturation pulses. For example, if a series of saturation periods of different lengths needs to be tested, but the total time of each experiment should be held constant (to maintain a consistent relaxation weighting for all scans), then the Presat Delay can be decremented as the saturation period is incremented. In the example below, the .Length of the saturation pulse is 500 ms.

Presat Delay = 0 ms, Number of Saturation Pulses = 6, and therefore total time = 3 sec

Presat Delay = 500 ms, Number of Saturation Pulses = 5, and therefore total time = 3 sec

Presat Delay = 1000 ms, Number of Saturation Pulses = 4, and therefore total time = 3 sec

Presat Delay = 1500 ms, Number of Saturation Pulses = 3, and therefore total time = 3 sec

Presat Delay = 2000 ms, Number of Saturation Pulses = 2, and therefore total time = 3 sec

Presat Delay = 2500 ms, Number of Saturation Pulses = 1, and therefore total time = 3 sec

Number of Sat Pulses = 10

NOTE: The total length of the saturation period is the .Length of a saturation pulse listed above, multiplied by the number of saturation pulses (See the "Number of Cest Pulses" parameter below). For example, a 600 msec .Length and Number of Cest Pulses = 10 is a 6 second saturation pulse. CAUTION: If respiration gating is used (Preparation Trigger is turned ON), then the pulse sequence will perform one ADDITONAL saturation pulse and then check the trigger. Therefore, set the number of saturation pulses to one less than the number that you actually want. For example, if you want to perform 10 saturation pulses at 600 msec for a total of 6 sec, and you want to use respiration gating, then set the number of pulses to 9, and 10 pulses will actually be performed. Note also that an 11th, 12th, etc. pulse may also be performed depending on the breathing of the mouse (see below for details). The Number of Sat Pulses must be at least 1. Therefore, the pulse program will perform at least 2 saturation pulses for the saturation period of a respiration-gated acquisition.

Sat Power = 4 uT

You can enter the saturation power in microTesla. The Saturation Power and the Saturation Pulse Length determine the flip angle of the saturation pulse:

Z degrees of the flip angle = (X saturation power in microTesla)(42.58 Hz/microTesla)((1 cycle/sec)/Hz)

(360 degrees/cycle)(Y .Length of 1 pulse in sec)

Or Z = (X)(Y)(15,328.8)

For example, 36,789.1 degrees = (4 uT)(0.6 sec)(15,328.8)

Note that if you change the Sat Power OR the saturation pulse length,

then the Saturation flip angle will be recalculated.

Sat Pulse Shape = bp32

bp32 is a block pulse. Other pulse shapes can be selected. Pulse shapes are stored in /opt/PV5.1/exp/stan/nmr/lists/wave/ . You can add other shaped pulses to this directory as needed.

Sat Pulse - expand this sub-menu

.Length = the length of one saturation pulse

.FlipAngle = the flip angle in degrees

.Attenuation = the attenuation value that sets the saturation power

The attenuation is determined from the flip angle and the calibration of the

90 degree pulse when you first click Traffic-Light.

All of the other parameters in this sub-menu can be ignored.

The "range" parameters establish the saturation frequency list. You can acquire ranges of the CEST spectrum with different intervals. For example, you can set three ranges, where the first range spans -12 to -3 ppm in 1 ppm increments, the second range spans -2.9 to +2.9 ppm in 0.1 ppm increments, the third range spans +3 to +12 ppm in 0.25 ppm increments, etc. The following parameters show how to set up this example:

number of ranges = 3 (note that the remaining parameters now have 3 boxes)

Begin Sat Freq range = -12 -2.9 3

End Sat Freq range = -3 2.9 12

Set Freq Interval = 1 0.1 0.25

number of Sat Freqs in range = 10 59 37

Note that the number of Sat Frequencies in each range is calculated from the other parameters. Do not enter numbers for the "number of Sat Freqs in range" because they will be recalculated. Instead, change the interval to change the number of sat frequencies in a range. Also, the interval CANNOT be zero. When you increase the number of ranges, the interval is zero by default. You must change this zero to a positive number.

Total Number of Sat Frequencies = the sum of "number of Sat Freqs in range" listed above (106 in this example)

Again, this parameter is not set by the user.

SatFrqArray = not set by the user. However, review the list to ensure that the saturation frequency list is correct.

Divide the number of a saturation frequency in Hz by the magnetic field strength

(i.e., 300 MHz for a 7T magnet).

Note that the exact Larmor Frequency of your magnet is used to set these

saturation frequencies, and the Larmor

frequency is often not exactly 300.000000 MHz.

Echo Time = the TE time, usually set as short as possible. However, TE cannot be set in cestRARE\_Pagel4.

Instead, set the effective echo time to be as short as possible.

Effective Echo Time = Echo time of the RARE, set this variable to be as short as possible.

Rare Factor = number of k-space lines to acquire after each excitation.

Repetition Time = This is the TR for the RARE acquisition, and should also be set as short as possible.

Number of Averages = 1. Values other than 1 will cause the pulse program to

repeat the saturation frequency and sum the acquisition with the previous acquisition.

Number of Repetitions = Not Used (automatically set to the total number of saturation frequencies).

Note that we have used the "repetition loop"

in the Bruker pulse sequence to iterate the saturation frequency.

Unlike cestFISP\_Pagel4, cestRARE\_Pagel4 CANNOT acquire a series of CEST spectra in one dataset.

Acquiring multiple spectra can be helpful for acquiring kinetics data, where a series of CEST spectra are continually acquired. However, in cestRARE\_Pagel4, I have hardwired NR and PVM\_NRepetitions to be the same value as NumSatFrqTotal. Therefore, NR cannot be changed. If you want to use cestRARE\_PAgel4 to acquire multiple CEST spectra, then edit parsRelations.c, remove the following line:

PVM\_NRepetitions = NumSatFrqTotal;

and then recompile (make clean; make all; and reinstall files as described above).

Then set Number of Repetitions to a multiple of NumSatFrqTotal.

Number of Echo images = 1

Estimated Total Scan Time = calculated INCORRECTLY.

However, the Scan Control menu shows the correct total scan time and time remaining.

Derive Pulse Gains = Yes

Encoding - expand this sub-menu

Phase Encoding Order = CENTRIC\_ENC

Use Centric Encoding so that the initial signal acquisition establishes the signal level for the image. The alternative is to use Linear Encoding. However, some CEST contrast is lost between the time that the acquisition is started at the edge of k-space, and the time that signal is acquired in the center of k-space. During this time, T1 relaxation occurs which reduces CEST contrast (T1 relaxation is the enemy of CEST). Therefore, use Centric Encoding. See "Dummy Echos" below for more details.

Preparation Options - Expand this sub-menu

Dummy Sans = 2

Setting Dummy Echos allows the magnetization to reach steady state before data is actually acquired. We use 8 dummy echos which seems sufficient for most samples. The Dummy Echos compensates for using Centric Encoding (described above).

Trigger Module= Set to OFF for no respiration gating.

Set to ON for respiration gating. If you select respiration gating, then the pulse sequence will perform the saturation period (10 pulses each for a 600 msec duration, for a 6 sec saturation period). Then the pulse sequence will perform ONE ADDITIONAL saturation pulse and then check to determine if the trigger is active. If the trigger is active, the pulse sequence will perform the RARE acquisition. If the trigger is not active, then the pulse sequence will perform 1 additional saturation pulse (600 msec in this example), and then check if the trigger is active. This loop continues until an active trigger is detected after a saturation pulse. For this example, the total saturation period is then 6.6, 7.2, 7.8, 8.4, etc., seconds. We use 6.6 seconds for our minimum saturation period which is very close to 100% steady state, so that additional saturation does not change the CEST signal. Use a Trigger Mode of "per\_PlaseStep" and a Trigger Delay of 1.000 ms. We use a SA Instruments respiration gating system, with "pulse invert" checked on, "Begin Delay" set to 200, "Max Width" set to 400, and "Gates" checked on.

IMPORTANT NOTE: Some Bruker scanners use "trignl1" and other Bruker scanners use "trigpl1". trignl1 appears to look for a negative change in voltage, while trigpl1 looks for a positive change in voltage, when the trigger is evaluated. This version sues "trignl1". I am testing other versions. - Marty Pagel, July 29, 2018

**How to use cestRARE\_Pagel4:**

1. Acquire a high resolution MR image of your mouse or sample. Multislice is OK for this image set.

2. Select the single image slice that you want to image with CEST MRI.

cestRARE\_Pagel4 only acquires single-slice data. Make a note of the isodist parameter for this slice

(i.e., slice position in millimeters).

3. Select New Scan and select cestRARE\_Pagel4

4. Use the Geometry Editor to Import Geometry from the high-resolution MR image acquired in step 1 above.

5. Set the number of slices to 1 and set the isodist to the value for the single slice that you

want to acquire (step 2 above).

6. Select your CEST parameters (See the notes above).

7. Ensure that your mouse or sample are at 37.0C plus or minus 0.2C

(plus or minus 0.5C may also be acceptable, but we set our tolerance to pulse or minus 0.2C).

8. Ensure that your mouse is breathing consistently and slowly. Note that you need a

~450 msec window with no lung motion for best results.

A breathing rate < 45 breaths per minute provides 1.33 seconds between breaths.

The mouse "gasps" for about 300 msec under anesthetic.

So you then have a ~1 second window to acquire your ~450 msec RARE acquisition. i

Faster breathing rates may not allow enough time for RARE acquisition between breaths.

9. Click the Traffic Light Icon. You are now acquiring cestRARE\_Pagel4 data.

CAUTION: A RARE acquisition can take a long time if you use a small RARE factor and/or set a large matrix. For example, a RARE factor of 4 and a matrix of 64 needs 8 excitations to acquire the 64 k-space lines. Also, cestRARE\_Pagel4 places a saturation period between each excitation. For example a 6-second saturation with 8 excitations will require 48 seconds just to acquire one image. For this reason, it is recommended that a large RARE factor and a small matrix be used. For example, a RARE factor of 64 and a matrix of 64 is a "single-shot" experiment that is much faster. Also, if there is motion between the excitations of a RARE that has a factor < matrix size, then this motion can cause artifacts in the image. For this reason, I personally prefer cestFISP\_Page4 rather than cestRARE.

**How to display a CEST Spectrum using Image Sequence Analysis.**

You can view your CEST spectrum in the Image Sequence Analysis tool, in the Image Display & Processing menu, Processing Submenu. In the Image Sequence Analysis tool, click File / More Buttons. A new row of buttons appears at the bottom of the ISA window. Click on Define ROI... on that bottom row. Define your ROI using the new ROI tool window that appears. In the ISA window, click on Function / Select ISA Function. Select the cest\_Pagel\_ISA function. Click on Calc. Points for ROI. A vertical line of dots will appear on the graph. Click on Initialize ISA. The CEST spectrum will appear. Note that the positive saturation frequencies appear on the right, while CEST spectra should be plotted with positive saturation frequencies on the left. Also, this procedure only displays a CEST spectrum to view the quality of your data. This tool does NOT analyze CEST spectra.

**Parameters specific to the CEST saturation period:**

PresatDelay = Delay before the saturation period

NumSatPulses = Number of Saturation Pulses in the Saturation Period.

All saturation pulses use the same pulse shape. A 10 microsecond delay is placed between pulses.

SatPower = Saturation power

SatPulseShape = Saturation Pulse Shape

SatPulse.Length = length of a single saturation pulse in milliseconds

SatPulse.Flipangle = the flip angle of the saturation pulse in degrees

Sat\_range\_number = number of saturation frequency ranges

Sat\_range\_start = start frequency of the saturation frequency range

Sat\_range\_end = end frequency of the saturation frequency range

Sat\_range\_interval = interval between saturation frequencies in the sat frequency range

Sat\_range\_NumSatFrq = Number of saturation frequencies in the range

NumSatFrqTotal = Total number of saturation frequencies

SatFrqList = The list of the saturation frequencies, in units of Hz.

**Advanced Notes about the pulse sequence timing:**

The standard RARE has the following start to the pulse sequence:

FQ1 - D4 - FQ8 - 10u - 5 u - 10u - FQ=o1list - D10 - alpha2 - 10u PO:sp3 - 10u - D4 - ...

To see this pulse sequence, see Spectrometer Control Tool / Tools / Pulse Program Tool...

The cestRARE pulse sequence with NO respiration gating

has the following modification after the second 10u and before the FQ=o1list

GATEP - D7 - FQ=o4list - 10u - {mtc - 10u - P27}

where a loop is denoted with {} brackets.

This additional sequence is listed in cestRARE\_Pagel4\_Trigger.mod

The ppg and 4ch files simply defer to this Trigger.mod file.

After the GATEP command, the Ds75 delay (also known as D[7]) is applied, which is the Presat Delay.

The pulse frequency is set to the value in the o4list, which takes 10u.

"mtc" is the start of the loop for the saturation pulse.

Then after another 10 usec delay, the P27 pulse is performed. This is the saturation pulse.

Then the sequence loops to MTC until the number of CEST pulses is reached.

Then RARE acquisition begins.

The cestRARE pulse sequence WITH respiration gating

has the following modification after the second 10u and before the FQ=o1list

GATEP - D7 - FQ=o4list - 10u - {mtc - 10u - P27} - 5u - {startgate - 5u - P27 - 30u - check\_trigger} - endgate - MORE

where MORE at the end is:

for a Avance III system, 5u delay to set trignl1, and 5 u delay to set GRADSYNC

for all other systems, 5u delay to set trigpl1

The first part of this pulse sequence is the same as the sequence with no respiration gating.

then after another 5 usec delay, another loop is performed, named "startgate".

This is an additional saturation pulse beyond the number of pulses requested in the menu.

See the "CAUTION" message above about the Number of CEST Pulses.

Then the trigger is checked during a 30u delay. This 30u is the shortest delay when checking a trigger.

If the trigger is active, then there 1 or 2 additional delays (depending on the version of Avance hardware,

and finally RARE acquisition begins.

If the trigger is NOT active, then the loop continues {5u - P27 - 30u}.

Note that this is an endless loop until the trigger is active.

**Files that need to be edited to add CEST to Bruker’s RARE acquisition method:**

Note that all edits in these files are annotated (except the parameter file cestRARE\_Pagel4).

Search for "Pagel" in a file and you will find these annotations.

BaseLevelRelations.c

cestRARE\_Pagel4 (this is the parameter file for a protocol. Edits are within the first 30 lines)

cestRARE\_Pagel4.4ch

cestRARE\_Pagel4.c (only 1 line needs to be edited)

cestRARE\_Pagel4\_INSTRUCTIONS (this file)

cestRARE\_Pagel4.ppg

cestRARE\_Pagel4\_Trigger.mod

initMeth.c

itenerary.txt

Makefile (directory paths have to be edited)

parsDefinition.h (at the end of the file)

parsLayout.h (near the end of the file)

parsRelations.c

Files that do NOT need to be edited but must be present:

callbackDefs.h

deriveVisu.c

gradTools.c

loadMeth.c

method.h

parsTypes.h

RecoRelations.c

relProtos.h

relProtos\_p.h

File for ISA function:

cest\_Pagel\_ISA (uses SatFrqList variable, and requires a line with cestRARE\_Pagel\_ISA as the function name).