Instructions for cestFISP_Pagel5, cestRARE_Pagel5, and cestUTE_Pagel5
for PV6.0.1 / Bruker Biospin MRI instruments

This cestFISP, cestRARE, and cestUTE MRI pulse programs from Bruker ParaVision 6.0.1 were developed by Marty Pagel with helpful collaborations, recommendations and example code from Ed Randtke, Kyle Jones, Guanshu Liu and Jiadi Xu. Although these pulse programs have worked on all Bruker MRI scanners with PV6.0.1 that we have tested, there is no guarantee that this pulse program will work on all Bruker scanners (in particular, the respiratory gating trigger may use different TTL logic on different systems). Also, while we are collegial researchers, we may not be able to provide assistance due to limited time and other priorities.

Version History
Note that previous versions used additional name prior to 2018.
cestFISP_Pagel1 = written December 2011 at the U of Arizona
cestFISP_Pagel2 = written May 2014 at the U of Arizona, includes respiratory gating
cestFISP_Pagel3 = written June 2018 at MD Anderson, includes a new menu layout
cestFISP_Pagel4 = written July 2018, with renamed variables and improved delays, and improved respiration trigger code
cestXXXX_Pagel5 = written May 2019, with new menu system for PV.6.0.1, and added RARE and UTE (where "XXXX" is "FISP", "RARE", or "UTE")

Installation instructions
1. Download and store the files in your home directory on your Bruker workstation. Alternatively, store the files in /opt/PV6.0.1/share, which is the default directory for shared methods.
2. Start ParaVision. Select the Workspace Explorer tab in the upper left of the screen. Then right-click on User Methods. Select Import/Source Method. Then change the directory to your home directory or /opt/PV6.0.1/share, and select cestXXXX_Pagel5.
3. To use the ISA tool to view CEST spectra on the Bruker workstation, copy the cestFISP_ISA_Pagel5, cestRARE_ISA_Pagel5, and cestUTE_ISA_Pagel5 files to /opt/PV6.0.1/prog/curdir/<your_login>/ParaVision/isa/src

How to use "cestXXXX_Pagel5" methods
After the code is installed, create an exam card (select File/New/Study). Then select the Explorer tab on the left side of the screen. Then select "Scan Programs & Protocols", and select User Methods. Left-click and drag the cestXXXX_Pagel5 method to the Exam card. Set up the FISP, RARE or UTE acquisition method as needed. Note that the scan time that is displayed in the menus and in the instruction list is an incorrect time (the total saturation time is not included in this total time). Most of the CEST parameters are on the CEST tab of the Contrast card (Figure 1). However, see the description about Averages and Repetitions, which includes a description about "Number of Sat Frq" and "Number of CEST Spectra" parameters on the Routine card.

Set the following parameters for CEST, on the CEST tab of the Contrast card. Note that Bruker menus have text boxes and buttons that do not always work smoothly when they are first used. You may have to enter and re-enter values until you get all of the parameters set.

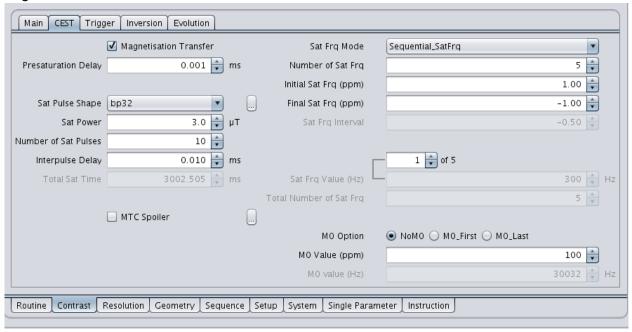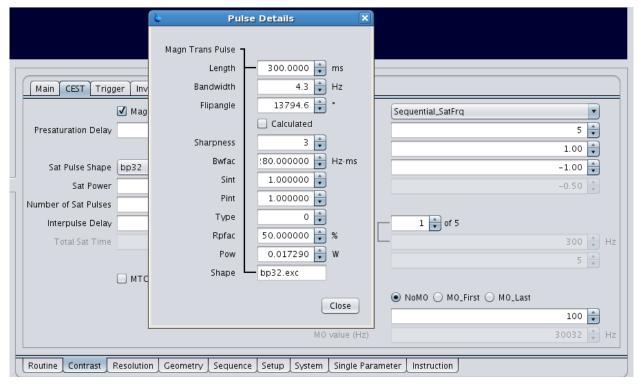Figure 1. The CEST tab in the Contrast Card.

Figure 2. The Pulse Details sub-menu in the CEST Tab

Magnetization Transfer: This button should be checked.

Presaturation Delay: This delay occurs BEFORE the saturation period, and does not have any saturation pulse. This delay is used when you want to test different saturation times with a series of CEST MR images, but you want the total TR time to be consistent for the set of images. For example, if you want to test 0.5, 1.0, 1.5, and 2.0 seconds of saturation time, but you want the total TR time to be 10 seconds (to avoid T1 relaxation effects, for example), then you would set the saturation pulse(s) to be a total of 0.5, 1.0, 1.5, and 2.0 seconds, and then set this Presaturation Delay to 9.5, 9.0, 8.5, and 8.0 seconds. Overall, this Presaturation Delay is rarely used, and is often set to 1 microsecond.

SATURATION PULSE PARAMETERS

Sat Pulse Shape: Select your desired pulse shape from the menu. Then immediately select the button to the right of the pulse shape text box. Select this button BEFORE you set the Sat Power value. Selecting this button shows the sub-menu with pulse parameters (Figure 2). Set the length of the pulse. The other parameters are automatically calculated and do not need to be changed. Note that the length of the pulse can be short, and then multiple pulses can be set (e.g., 300 msec pulse, 10 pulses, for a total of 3 seconds of saturation time), which is especially important for respiratory gating.

Sat Power: Set this value in microTesla. To verify that the saturation pulse parameters are correct, return to the button to the right of the Sat Pulse Shape text box. Re-enter the length of the pulse if needed. As described above, you may have to enter and re-enter values until you get all of the parameters set.
Special Note: The Flipangle of the pulse is calculated using the following equation:
Z degrees of the Flipangle = (X saturation power in microTesla) (42.58 Hz/microTesla) ((1 cycle/sec)/Hz) (360 degrees/cycle) (Y Length of 1 pulse in seconds)
Or Z = (X) (Y) (15,328.8)
For example, a 3 uT pulse for 300 msec is a flip angle of 13,794.6 degrees, as shown in Figures 1 and 2.

Number of Sat Pulses: The number of pulses in the saturation period. For example, if you set the Number of Sat Pulses to 10, and the Sat Pulse Length is 300 msc, then the method will apply a total of 3 seconds of saturation.

Interpulse Delay: The delay between pulses during a multipulse saturation period. This value is typically 10 microseconds.

Total Sat Time: This value is calculated by the method, and not entered by the user. This parameter is the time of the total saturation period.

MTC Spoiler: Check this box if you want a short spoiler to be performed immediately after the saturation period. We do not use a spoiler because we have not seen a difference in quantitative CEST MRI results with or without a spoiler. Also, we often perform respiratory gating, and we want to reduce the time between the end of the saturation period and the start

of acquisition so that the total acquisition can occur with no lung motion (i.e., it can be difficult to acquire the data before the next breath starts, if the breathing rate is not slow). However, other CEST MRI researchers prefer a spoiler.

SATURATION FRQUENCY LIST

Sat Frq Mode: The mode can be set to Sequential_SatFrq, Interleaved_SatFrq, From_File, or Enter_Manually.
- The "Sequential_SatFrq" and "Interleaved_satFrq" options use the Number of Sat Frq, Initial Sat Frq, and Final Sat Frq parameters below.
- If you select the "From_File" option, then a text box appears, which you can use to enter the name of a file in your home directory. This file should have values in ppm that are sequentially used for saturation, with one value per line and with no punctuation and no blank lines (including no blank lines at the end of the file). The method will read these ppm values, and convert to Hz values based on the SFO1 Larmor Frequency of your magnet. We prefer to use a frequency list, because we can then define sub-ranges in a CEST spectrum with different step sizes. For example: -10, -8, -6, -4, -3, -2, -1, -0.5, -0.25, 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 8, 10, 100.
- If you select "Enter_Manually", then the "Sat Frq Value (Hz)" box changes from gray to white and you can manually enter each value in Hz.
Note that the final frequency list in Hz is stored in the files for your dataset, specifically in the methods file, immediately after the line that lists "##$PVM_ppgFreqList1", regardless of the Sat Frq Mode that you select. Therefore, the final frequency list for a dataset is stored within the dataset.

Number of Sat Frq: The number of saturation frequencies

Initial Sat Frq (ppm): first value, typically the lowest or most negative value. However, the initial value can be the highest or most positive value, and the Final Set Frq can be the lowest or most negative value. You can list frequencies either from low-to-high or high-to-low.

Final Sat Frq (ppm): last value, typically the highest or most positive value

Sat Frq Interval: Cannot be entered by a user, this step size is calculated based on the three previous parameters.

Sat Frq Value (Hz): The saturation frequency in units of Hertz, for the counter number that is listed above this parameter

Total Number of Sat Frq: The total number of saturation frequencies. This number is either the Number of Sat Frq listed above, or (1 + Number of Sat Frq) if the M0 option is selected to add a M0 value (see below).

M0 Option: This option allows you to add an extra saturation frequency that that has a very large value (either a positive or negative value), which is used to measure the "M0" MRI signal that should represent no direct saturation of water or the source of CEST (exogenous contrast agent, or endogenous protein or metabolite). Selecting "NoM0" does not include this extra saturation frequency. Selecting "M0_First" or "M0_Last" will add the M0 frequency to the start or end of the list, respectively. We prefer to use a frequency list without an M0 option, and we define the M0 frequency in that list.

M0 Offset (ppm): Enter the value in PPM for the M0 offset.

M0 Offset: The calculated value in Hz, based on the SFO1 Larmor frequency of the magnet

A note about Averages and Repetitions:
The Averages parameter that is part of the standard FISP sequence can be used, but with caution. This parameter is listed on the "Routine" tab (Figure 3). For example, if the number of averages is set to 2, and there are 3 saturation frequencies at 10, 0, and -10 ppm, then the method will perform 6 image acquisitions in this order: 10, 10, 0, 0, -10, -10 ppm. Then the reconstruction routine will take the average of the first 2 images at 10 and 10 ppm sat frequency, the second 2 images at 0 and 0 ppm, and the third 2 images at -10 and -10 ppm. This method will NOT acquire image acquisitions in this order: 10, 0, -10, 10, 0, -10 ppm.

If you want to acquire images in the order 10, 0, -10, 10, 0, -10 ppm, effectively acquiring individual CEST spectra and then averaging the spectra, then use the "Number of CEST Spectra" parameter on the Routine card. For example, set the CEST frequency list to 10, 0, and -10 ppm, and then set the "Number of CEST Spectra" to 2. Then 6 images will be acquired with saturation at 10, 0, -10, 10, 0, -10 ppm. Then a Matlab script can be used to take the average of the 1st and 4th images (the two images acquired with 10 ppm saturation), the 2nd and 5th images (the two images acquired with 0 ppm saturation) and the 3rd and 6th images (the two images acquired with -10 ppm saturation). Also, you can use the "Number of CEST Spectra" parameter to acquire a series of CEST spectra over time, to observe the evolution of the CEST signal (e.g., during pharmacokinetics studies, or enzyme activity studies). Note that the Routine card also lists the "Number of Sat Frq" saturation frequencies for 1 CEST spectrum. This parameter is the same parameter as listed on the CEST tab of the Contrast card, and just displayed on the Routine card for clarity. Then the "Repetitions" parameter on the Routine card is the "Number of Sat Frq" multiplied by the "Number of CEST Spectra", and the "Repetitions" value cannot be changed by the user.
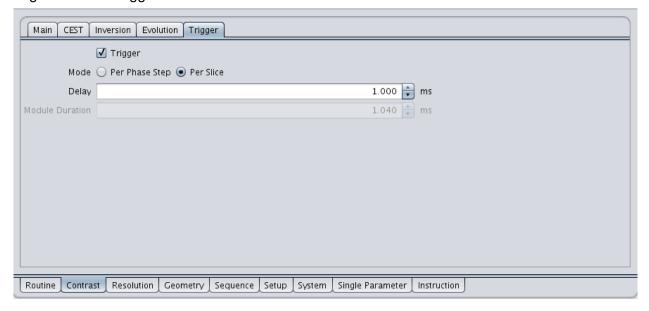
Figure 3. The Routine card.

A note about respiratory gating:

To set up respiratory gating, select the Contrast Tab and then select the Trigger tab (Figure 4). Check the Trigger box, and select "Per Slice". We use a "single-shot" (aka single-excitation) FISP, uTE, or RARE acquisition (with a RARE factor that matches the phase dimension), because acquiring a series of images with a range of saturation frequencies takes too long if multiple "shots" are needed to acquire each image. Therefore, if we use "single-shot" acquisitions, the triggering is "Per Slice" and not "Per Phase Step". Also, the respiratory-gated saturation period in our code is only set up for "Per Slice" gating.

Figure 4. The Trigger tab in the Contrast card.

The pulse sequence will perform the saturation period with the number of saturation pulses that you enter for "Number of Sat Pulses" (see above). Then the pulse sequence will apply one additional saturation pulse and then check if the respiratory trigger is active. If the trigger is active, then the acquisition will start. If the trigger is inactive, then the pulse sequence will once again perform one additional saturation pulse, and then check if the trigger is active. This loop continues until an active trigger is detected after a saturation pulse.

Importantly, you should use many short saturation pulses for the saturation period, rather than use a single long saturation pulse, because the extra saturation pulse added in cases when the trigger is still inactive has the pulse length for the pulse(s) before the trigger is checked. Also, you should set the number of pulses to be one less than the total number that you want, assuming that the trigger is active when the trigger is first checked. For example, if you want to apply 20 pulses that are 300 msec for a 6 sec saturation period, then set Number of Pulses to 19, and turn on the Trigger. Then you will get 20 pulses, or 21, 22,… pulses until the trigger is active, where 20 pulses = 6 sec, 21 pulses = 6.3 sec, 22 pulses = 6.6 sec, etc. In practice, and if the animal has a good, steady respiration profile, then the trigger is typically active at 6.0, 6.3, or 6.6 seconds. On occasion, an entire breath is missed, and the saturation can be very long before the next trigger is active. For this reason, we typically use a very long saturation time (6 seconds), because CEST is typically at steady-state by 6 seconds, and adding more saturation does not increase the CEST signal.

Note that triggering at the START of saturation period (rather than the END of the saturation period in our method) only works reasonably well if you have a very short saturation period (e.g., ≤500 msec with very slow breathing). Otherwise, a longer saturation period causes the entire saturation-acquisition process to be too long, and the next breath occurs before the acquisition is completed. Also, for long saturation periods such as 3 seconds, triggering the pulse sequence to start and then applying a 3 second saturation before acquisition would cause the acquisition to be asynchronous with breathing (unless your mouse breaths once every 4+ seconds, but that breathing rate is too slow for the mouse to survive). Therefore, our method of triggering at the END of the saturation period allows the acquisition to be synchronous with breathing motion.

The cestUTE_Pagel5 method has some limitations with regard to respiratory gating. First, you need to acquire a standard UTE image with the Bruker UTE method, with "Automatic Setup + Acquisition" to adjust the gain, etc. Then you can use the cestUTE_Pagel5 method with trigger on, but you cannot perform an automatic setup. Instead, select the Instruction card, then click and drag "GOP" from the right box to the left box, and select "Individual setup / Acquisition" to acquire data without the initial automatic setup. Also, the cestUTE_Pagel5 method on our MRI scanner requires a 50 microsecond delay at the end of the last saturation pulse, before the UTE image is then acquired. The cestFISP_Pagel5 and cestRARE_Pagel5 methods only require a 30 microsecond delay between the end of saturation and start of acquis ion. We are currently testing and improving cestUTE_Pagel5, so that future versions may have better timing.

Also, the cestUTE_Pagel5 method does not use dummy scans, regardless of whether trigger is used or not used. Performing dummy scans after the saturation period will decrease CEST contrast. Performing dummy scans before a long saturation period is not useful because the dummy scans to not immediately precede the acquisition of the image. Again, we are currently testing and improving cestUTE_Pagel5, so that future versions may have better performance.

A note about how to display your CEST spectrum on the Bruker workstation:

You can view your CEST spectrum in the Image Sequence Analysis tool, in the Image Display tool that was used with ParaVision version 5.1. See the installation notes to install the cestFISP_ISA_Pagel5, cestRARE_ISA_Pagel5, and cestUTE_ISA_Pagel5 files.

1. Select the Pallet/Explorer tab, and then click on the Datasets button.
2. Right-click on the data set that you want to analyze. Select "View in Image Display".
3. Select the Processing menu, and select "Image Sequence Analysis…".
4. Click on "File" and select "More Buttons".
5. Click on "Define ROI…" at the bottom left of the screen. Define your ROI (for example, select New, select Circle, draw a circle on your image, and select OK). Be sure to click on the name of the RI in the ROI tool to select the ROI that you want to use for subsequent steps.
6. Click on "Calc. Points for ROI" at the bottom of the screen. A vertical line of dots will appear on the graph.
7. Click on Initialize ISA and select cestFISP_ISA_Pagel5, cestRARE_ISA_Pagel5, or cestUTE_ISA_Pagel5. The signal in your ROI will be plotted as a function of saturation frequency.
8. If you included a M0 saturation frequency that is at a very large value, then you may want to remove this data point to more clearly view your spectrum. Select the row of the M0 data point in the table above the graph. Then click on Table, "Remove selected points". Then click on "Initialize ISA" to redraw your graph without this point at the M0 frequency.

Note that CEST spectra should be plotted with positive-value saturation frequencies on the left of the x-axis, but the ISA tool plots these positive-value frequencies on the right of the x-axis. Also, this procedure only displays a CEST spectrum to view the quality of your data. This tool does not analyze CEST spectra. This tool is helpful "quick check" to ensure that you have a good CEST MRI dataset during your acquisition session.