Version 7.0

Application Guide: Local Cerbral Glucose Utilization

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Contents

Introduction

This document is a supplement to the **MCID™ Basic** online Reference Manual. It describes how use **MCID Basic** to quantify rates of local cerebral glucose utilization (LCGU) in tissue sections that have been exposed to autoradiographic film. It presumes that standard experimental procedures have been followed.

LCGU can be calculated with Sokoloff's (Sokoloff et al., 1977) original operational equation (Equation 1) or with the Savaki (Savaki et al., 1980) modification (Equation 2). The Savaki equation should be used when plasma glucose concentrations vary considerably during the experiment (within a range of about 70 - 250 mg%) especially when the animal becomes progressively hyperglycemic. Keep in mind that the lumped constant changes, increasing in hypoglycemia and decreasing in hyperglycemia (Schuier et al., 1990; Suda et al., 1990).

Equation 1: *Sokoloff's original operational equation for LCGU.*

$$R_i = \frac{C_i^*(T) - k_1^* e^{-(k_2^* + k_3^*)T} \int_0^T C_p^* e^{(k_2^* + k_3^*)t} dt}{\left[\frac{\lambda \cdot V_m^* \cdot K_m}{\Phi \cdot V_m \cdot K_m^*}\right]\left[\int_0^T \left(\frac{C_p^*}{C_p}\right) \mathrm{dt} - e^{-(k_2^* + k_3^*)T} \int_0^T \left(\frac{C_p^*}{C_p}\right) e^{(k_2^* + k_3^*)t} dt\right]} \quad \mu mol/100g/min$$

Equation 2: *Modified operational equation for LCGU.*

$$R_i = \frac{C_i^*(T) - k_1^* e^{-(k_2^* + k_3^*)T} \int_0^T C_p^* e^{(k_2^* + k_3^*)t} dt}{\left[\frac{\lambda \cdot V_m^* \cdot K_m}{\Phi \cdot V_m \cdot K_m^*}\right] \int_0^T \left[\frac{(k_2^* + k_3^*) e^{-(k_2^* + k_3^*)t} \int_0^T C_p^* e^{(k_2^* + k_3^*)t} dt}{C_p(0) e^{-(k_2 + k_3)t} + (k_2 + k_3) e^{-(k_2 + k_3)t} \int_0^T C_p e^{(k_2 + k_3)t} dt}\right] dt}$$

Ri is the rate of glucose utilization (μmol/100g/min.). *T* is the time at the termination of the experimental period. *Ci** is the total [^{14}C] concentration in a single homogeneous tissue of the brain. *Cp** and *Cp* represent the concentrations of [^{14}C]deoxyglucose and glucose in the arterial plasma. λ is the ratio of the distribution space of deoxyglucose in the tissue to that of glucose. Φ is the fraction of glucose which, once phosphorylated, continues down the glycolytic pathway. *Km**, *Vm**, and *Km*, *Vm*, represent the Michaelis-Menten kinetic constants of hexokinase for deoxyglucose and glucose, respectively. The six constants collectively constitute the lumped constant.

Basic Procedure

The basic procedure for quantifying rates of glucose utilization in autoradiographic images is as follows:

1. Create an LCGU **Accessory file** for every animal used in the experiment. An accessory file contains data obtained from blood samples collected over the course of the experiment.

2. Adjust camera magnification and focus.
3. Establish a **Flat Field Correction** to correct for uneven illumination.
4. Establish an LCGU calibration for the first animal used in the experiment. The calibration is based on accessory file data and the values of the [^{14}C] concentration standards.
5. Sample any/all specimens associated with the LCGU calibration (i.e., same animal, same film).
6. Repeat Steps 4-5 for all remaining animals. Re-calibration is necessary whenever you change animals and/or films.
7. Summarize and save the data.

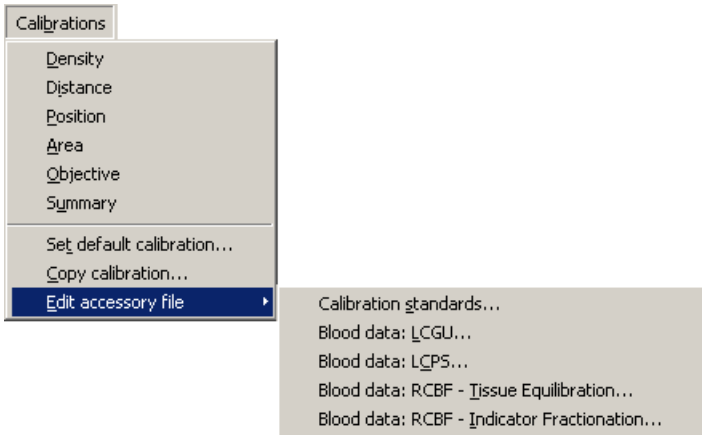
Step 1: Creating Accessory Files

Accessory file data are necessary to solve the operational equations for determining rates of local cerebral glucose utilization. The file contains data obtained from blood samples collected over the course of an experiment (LSC counts and glucose concentrations), and are used to calculate various parameters and integrals that are contained in the operational equation. A separate file must be created for every animal that is used in the experiment.

To create an LCGU accessory file, go to the **Calibration** operational window:

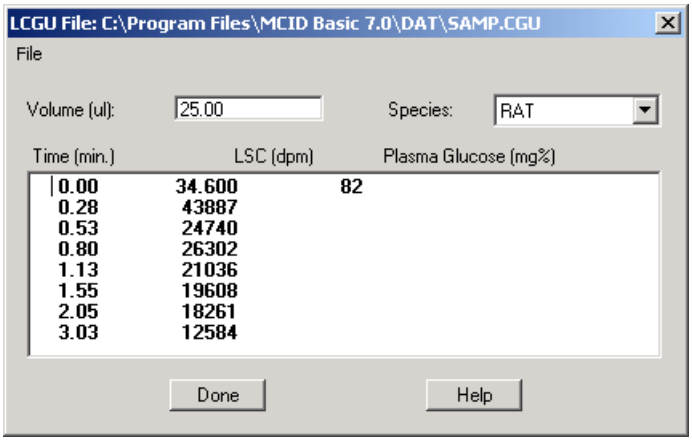
Open the *Calibrations* menu and select *Edit accessory file > Blood data: LCGU* (Figure 1). An *LCGU* dialog box is displayed.

Figure 1: *The Calibrations > Edit accessory file menu option is used to create standard plasma data files for local cerebral glucose utilization (LCGU).*



In the *LCGU* dialog box, enter the **Species**, sample **Volume (µl)**, sample **Times (minutes), LSC (dpm)** values, and **Plasma Glucose (mg%)** values. Use the <**Tab**> key to move the cursor laterally within the table. The first LSC value must be a time 0 baseline count (Figure 2).

Figure 2: *A typical accessory file used for LCGU analysis.*



If any blanks appear in the glucose column, **MCID Basic** interpolates the missing values when it solves the operational equation. If the final time has no LSC and glucose value, it is assumed to be a kill time and values are extrapolated. When you have entered all of the necessary data, use the *File* menu within the dialog box to save the file. **MCID Basic** will automatically attach a *.cgu extension to the specified filename.

Step 2: Adjusting Magnification and Focus

Magnification is controlled by moving the camera up or down. Place a sheet of film on the light box and adjust the camera height while digitizing. With a 55mm lens, you should be able move the camera close enough so that a coronal rat brain section will almost fill the entire field of view. If the camera is too close to the specimen, you will not be able generate a clearly focused image.

If higher magnification is required (e.g., for mouse brain or other small tissue sections), use extension tubes. Extension tubes are little barrels that connect between the lens and the F-C adapter. They function like a bellows to increase magnification of the lens. Various sizes of extension tube give different magnification factors. For example, coronal section of spinal cord can be made to fill the screen when all three tubes are used.

Step 3: Establishing a Flat Field Correction

Camera images typically exhibit some intensity variation over the field of view due to uneven illumination. This “shading error” requires correction to less than 1% if accurate densitometry is to be performed. The **Flat Field Correction** compensates for shading error.

TO ESTABLISH A FLAT FIELD CORRECTION:

1. Remove the film from the field of view.
2. Select a pseudocolor look-up table (i.e., SPECT2.VIS).
3. Adjust the illumination intensity on the light box so that a blank field of view appears bluish-pink. Be sure to use a true blank field, not film background!

4. Ctrl-click on the **Digitize** icon and select **Flat Field Correction** from the list of *Input Device Operations*. If the operation is not present, click the **[Customize]** button to add it to the list.
5. A *Flat Field Correction* dialog box will appear (Figure 3). An **[Acquire]** and **[Clear]** button are displayed at the bottom of the dialog box, but only one of them will be active. If the **[Clear]** button is active, it means that a flat field correction is already in effect. Press **[Clear]** to remove it.
6. Select **Digitize** as the correction source and **Pixel by pixel** as the correction method.
7. The **Frame average** option will reduce the contribution of random electronic noise to the shading error. Three or four will usually do, unless you disable the **Smoothing** and **Median** filters (see below).
8. By default, a **Smoothing** and **Median** filter is applied to the shading error reference image (i.e., the blank field image) to reduce spatial variation and improve the signal-to-noise ratio. If you choose to disable these filters, be sure to use the **Frame Averaging** option when establishing the flat field correction, and increase the number of frames to 16 or more.
9. Press **[Acquire]** to establish a flat field correction.
10. **MCID Basic** scans the blank field and determines how much each pixel deviates from the mean gray level value. Subsequent images are automatically corrected for uneven illumination, based on this error pattern.
11. Click **[OK]** to exit the dialog box.

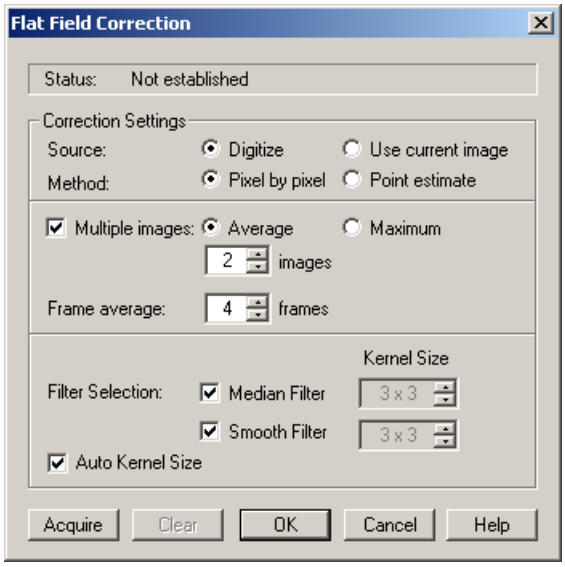
Please note, the image that you see after you exit the dialog box is not a corrected image. The **Flat Field Correction** is not applied until the next time you digitize. If you wish to see the effects of the correction, digitize the same blank field again. The image will 'even out' as soon as digitization is terminated.

Once established, the flat field correction becomes part of the camera's default configuration. Unless disabled or modified, **MCID Basic** will apply the same correction each time the particular camera is used, even if you have exited and re-started **MCID Basic**. As such, you should establish a new **Flat Field Correction** at the beginning of each analysis session.

One should also re-establish a **Flat Field Correction** whenever there is a change in ambient lighting, camera position, focus, camera gain and offset, light box position, or any other major factor that might affect the illumination pattern. Small changes in illumination level do not usually need a new correction, however, providing that optical system components are not moved relative to each other.

To remove or modify the current flat field correction, click the **[Clear]** button on the *Flat Field Correction* dialog box. Press **[Acquire]** to establish a new one.

Figure 3: *The Flat Field Correction dialog box.*

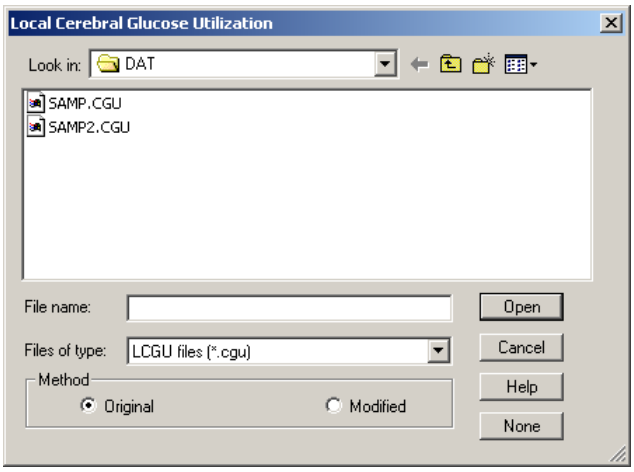


Step 4: Establishing an LCGU Calibration

The goal of an LCGU calibration is to construct a standard curve that relates optical density (ROD) to rates of glucose utilization (µmol/100g/min). The calibration is calculated from i) data contained in a single accessory file; ii) the nCi/g values of the [^{14}C] standards, and iii) the ROD values of the [^{14}C] standards when they have been exposed to film. A set of [^{14}C] standards should be present of every sheet of film. A separate LCGU calibration must consequently be established for each animal and each sheet of film.

To create an LCGU calibration, go to the **Calibration** operation window and select *Calibrations* > *Density*. Change the density unit to **LCGU**. A dialog box now appears (Figure 4), asking you to select an accessory file (*.cgu) and the method (**Original** or **Modified**) for calculating LCGU. Once you have selected an accessory file and exited from the dialog box, **MCID Basic** will then prompt you to select a density standards file. Pick the [^{14}C] standards file (calibrated in nCi/g) if you have one. Otherwise click **[None]** to proceed.

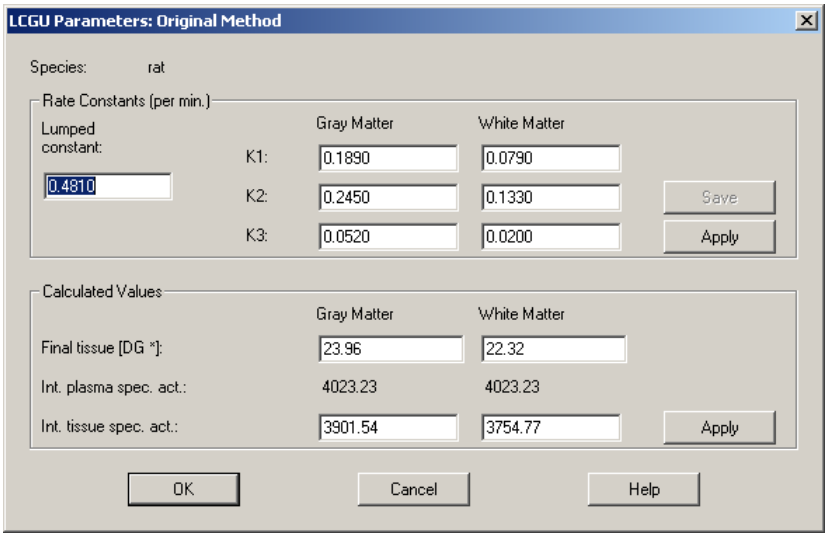
Figure 4: *Select an accessory file and a method for calculating LCGU values.*



An *LCGU Parameters* dialog box appears next (Figure 5), which displays a variety of parameters that will be used in solving the operational equation. **MCID Basic** uses appropriate default values for **Rate** and **Lumped constants** in the species whose name is read from the accessory file. **Calculated Values** are derived from the data contained within the selected accessory file. Note that separate values are given for **Gray Matter** and **White Matter**.

Changes to any terms in the LCGU equation may be made by entering new values into any portion of the solution screen. For example, the lumped constant is sensitive to the plasma glucose level and should be modified if hypo- or hyperglycemia is present. Enter any relevant changes to the default constants and click **[Apply]** to re-calculate the parameter. It is also possible to skip the accessory file entirely and to type in pre-calculated values for the plasma integrals. Please note that any changes made by editing the **Rate** or **Lumped constants** are temporary. That is, the default values will be used the next time the equation is solved.

Figure 5: *The LCGU Parameters dialog box.*



The next step is to calibrate to [^{14}C] concentration standards. In an LCGU experiment, this parameter represents the total [^{14}C] trapped in the tissue at the end of the experiment, Ci*.

The goal of this calibration is to create a curve that relates the system's internal density measurement units (ROD) to the [^{14}C] concentration values (nCi/g). This is achieved by entering the concentration values into a calibration table and reading the density of each corresponding standard. As each standard is sampled, **MCID Basic** plots the standard curve (density vs. standard value) next to the calibration table. The standard curve should span the entire range of densities found in the specimens under study. At least one set of standards should be also present on each film.

The general procedure is as follows:

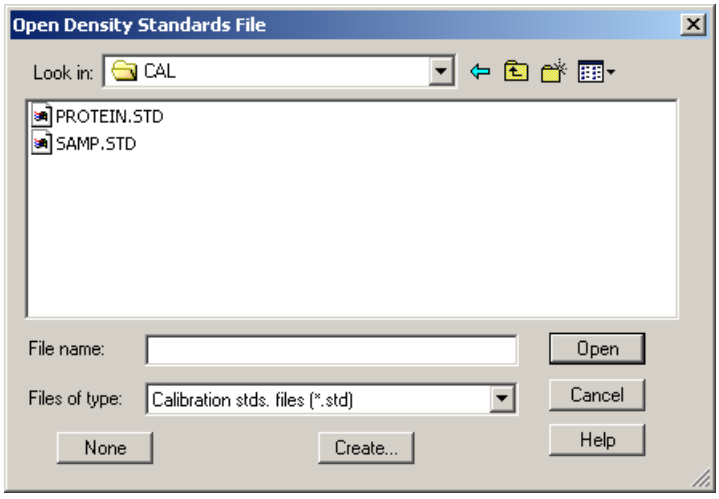
1. Digitize the standards. Adjust the illumination level so that film background appears pinkish-blue with the SPECTRAL.VIS or SPECT2.VIS look-up table. This step ensures that you are maximizing the dynamic range of the system.
2. Construct a calibration curve. Enter the [^{14}C] standard values into the calibration table and sample the standards.
3. Select a curve fitting function.
4. Select an extrapolation function. This step tells **MCID Basic** what to do with pixels that are outside the range of calibration values.

Building the Calibration Table

Entering the Standard Values

When you click on **[OK]** to leave the *LCGU Parameters* dialog box, you asked to select a density standards file (i.e., a file containing the [^{14}C] concentrations; see Figure 6). To load a file from disk, select the file and click on **[OK]**. If you want to type the values into the calibration table, click on **[None]**. To create a standards file, click on **[Create]** (see the Advanced Details section for instructions).

Figure 6: *The Open Density Standards File dialog box.*



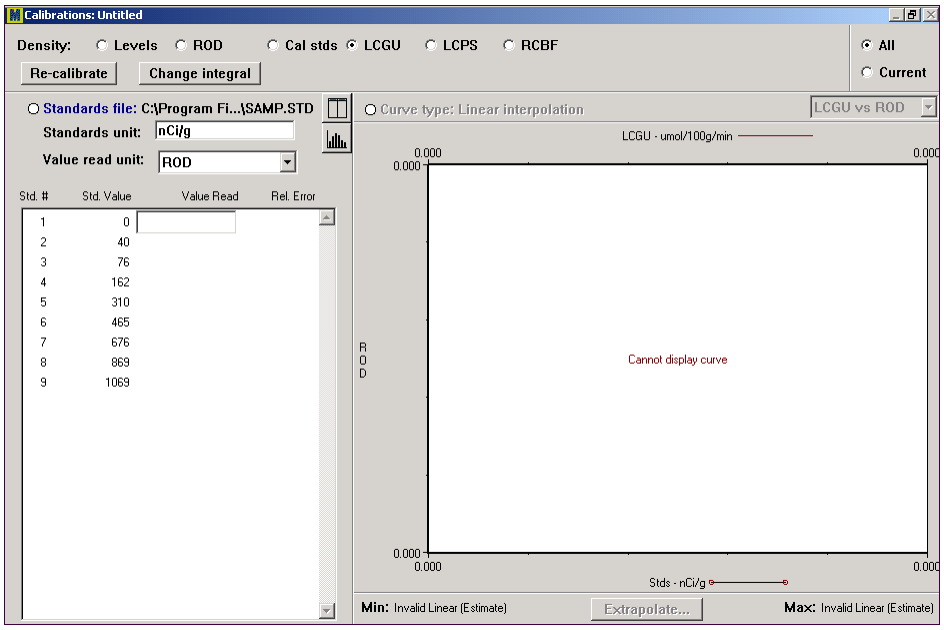
The **Calibration** window now appears as it does in Figure 7. On the left side of the window is a table with four columns labeled **Std #**, **Std Value**, **Value Read**, and **Rel Error**. Just

above the table are two entry fields, one for the **Standards unit** and one for **Value read** unit. To the right of the table is the region where the calibration curves will be plotted as the standards are read.

If you are using a density standards file, the standard values are displayed in the **Std Value** column. Otherwise, type the values of the standards into this column and enter the appropriate concentration units (nCi/g) into the **Standards unit** entry field. We recommend using a "0" as the lowest concentration standard. When you have entered all of the standard values, click the cursor in the **Value Read** column next to the first standard value.

> **Note:** When entering the standard values, make sure you use 'tissue equivalent' values and that they are appropriate for the thickness of your tissue sections. The units must be in "nCi/g".

Figure 7: *The Calibration window.*



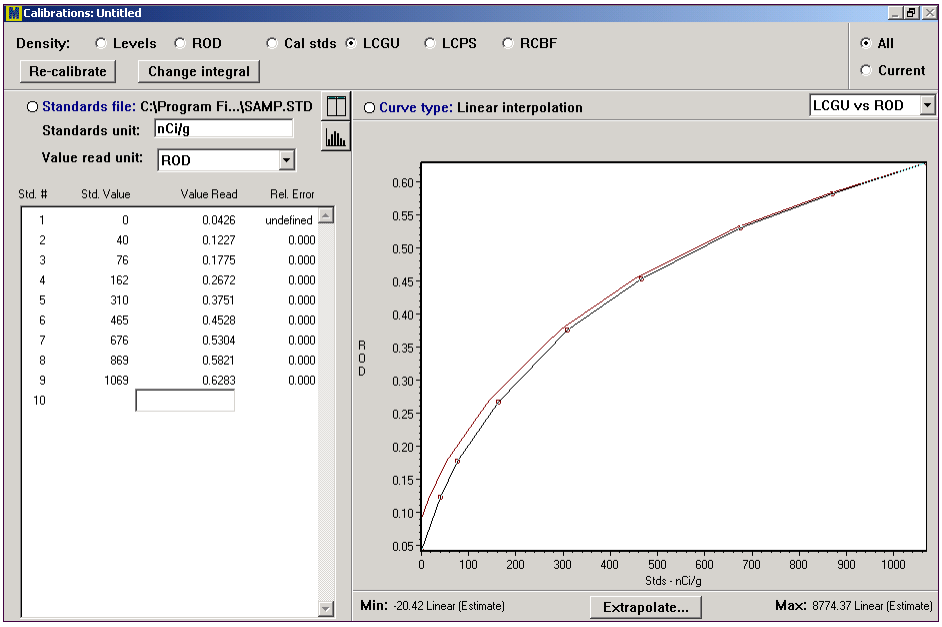
Sampling the Standards

Select a pseudocolor look-up table (SPECTRAL.VIS or SPECT2.VIS) and digitize the standards. A pseudocolor display makes it easier to see the appropriate light intensity settings for background and standards. It is important to optimize the lighting of your specimen. Optimal lighting varies with film density but, ideally, there is a range of less than 1 ROD between the darkest and the lightest areas of the film. In this case, blank film would read about 0.05 ROD in an 8-bit image (appears pinkish blue in the pseudocolor image), while the darkest areas of the autoradiograph read about 0.9 ROD (appear dark red). Do not read from film areas that are so light that **MCID Basic** sees a maximum light value (white). If the film background is pink with many white speckles (saturated pixels), turn down the illumination a bit.

> **Note:** Small changes in illumination level do not usually require a new **Flat Field Correction**, providing that optical system components are not moved relative to each other.

The lightest standard should be a film background reading, entered as a zero concentration in the **Std Value** column. The darkest standard read should lie within a fairly linear region of film response. Starting with the lightest standard, sample each one in order of ascending concentration. Re-digitize and re-position the standards as needed (do not change the camera height). Use the **Box** sample tool, and adjust its size so that it fits just within the edges of the standard. As each standard is sampled, its density value appears in the calibration table in the **Value Read** column, and the entry field automatically shifts to the next position on the list. The density calibration curve (ROD vs. nCi/g) is plotted to the right of the calibration table. Since [^{14}C] concentration is the only remaining parameter required to solve the operation equation, **MCID Basic** plots the LCGU calibration curve as well (Figure 7). The LCGU values (µmol/100g/min) can be plotted as a function of ROD or relative to the standard values.

Figure 8: *A completed LCGU calibration.*



Curve Fitting

Once you have constructed the calibration table, you need to fit a curve to your data points. Use the **Curve type** option to select an interpolation or approximation function that calculates density values that lie between the steps provided by the calibration reference (Figure 8).

The graph shows how well a selected fitting function fits the concentration values of the reference. A numerical estimate of goodness of fit is available from the **Rel Error** column in the calibration table, where:

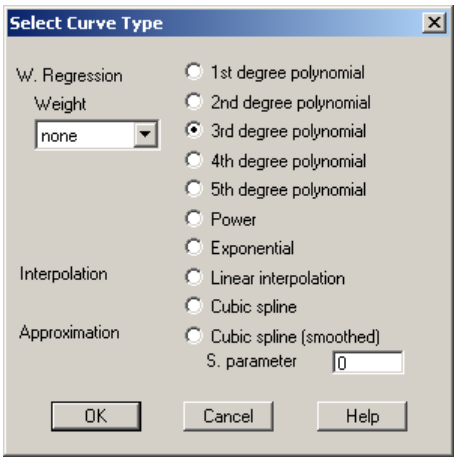
Relative Error = (standard value - curve value) / standard value

With any good fit, the error proportions will be low (< 0.1).

Experiment with all of the fitting functions and select the one that provides the best fit (e.g., where the **Rel Error** for all points is < 0.1). One of our own favorites is the smoothed spline, which offers significant advantages relative to the unsmoothed spline. The unsmoothed

spline is a cube root function that goes through all the points. Because it accommodates to standards that do not fit a cube root polynomial, the unsmoothed spline may contain abrupt changes in slope. Smoothing the spline allows it to deviate from all of the points, except for the lightest density reference (the X,Y origin of the calibration). The smoothed spline is forced through the lightest reference.

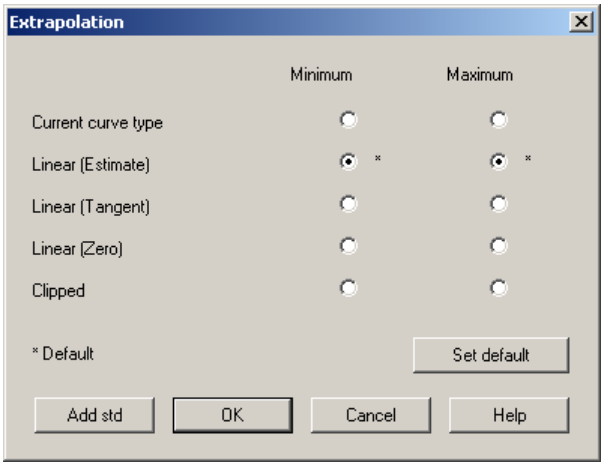
Figure 9: *Interpolation or approximation functions are used to calculate concentration values that lie between those contained in the standard set.*



Extrapolation Functions

By default, **MCID Basic** extrapolates calibrated values for pixels that are out of range (i.e., that do not fall within the calibration curve). A variety of extrapolation functions are available, and they can be applied to either end of the calibration curve. Click on the **[Extrapolate]** button located at the bottom of the calibration curve. This displays the *Extrapolation* dialog box, in which you can either select different forms of extrapolation or turn extrapolation off (i.e., clip pixel values to the minimum or maximum standard value).

Figure 10: *Density values beyond the range of standard values are estimated by extrapolation of the standard curve. You can apply different functions to the maximum and minimum ends of the curve.*



Validity of LCGU Calibrations

Density calibrations are valid only under a narrow range of conditions. ROD values, for example, will fluctuate in response to changes in illumination, lens aperture settings, camera height, camera settings (gain, offset, exposure time) and even to changes in ambient lighting conditions. ROD values will also fluctuate from one sheet of film to the next.

An LCGU calibration is consequently valid under the following conditions:

1. Sections must be from the animal associated with the current accessory file.
2. Sections must be on the same sheet of film as the standards.
3. Sections must be imaged under the same optical conditions that were in effect when the calibration curve was established.

Re-calibration is necessary whenever any of these conditions change.

Step 5: Sampling the Specimen

Once you have calibrated ROD to nCi/g, you are ready to gather data from the autoradiogram. When you return to the **Sample** operation window, you will see a column labeled "Dens - µmol/100g/min". To gather data, select a **Sample** tool and click it on the image (see *Chapter 4: Collecting Data* in the online **Reference Manual** for complete descriptions of **Sample** tools and instructions for their use).

At the top and to the right of the data table you will see the letters "**GM**" next to the current channel number. This means that **MCID Basic** is using the **Gray Matter** constants when it calculates µmol/100g/min. from the operational equation. Press the **<F11>** key to use **White Matter** constants ("**WM**"). Make sure to use **GM** constants when sampling from gray matter and **WM** constants when sampling from white matter.

If necessary, use **Visuals** controls to make features of interest more visible when sampling. You can change the way an image looks by selecting a different look-up table (LUT), by enhancing contrast, or by changing the LUT mapping function. Although these Visuals manipulations change the way an image looks, please remember that none of them change the actual density values of the image pixels. See the online *Reference Manual* for details (*Chapter 12: Altering Image Appearance*).

Calculating the Data Table Value

LCGU data are reported in the data table in calibrated units (µmol/100g/min.). **MCID Basic** calculates the value by first calibrating each pixel contained in the sample and then taking the mean of the calibrated pixel values. This is called "integrated optical density", or IOD:

Integrated Optical Density = Sum of the calibrated pixel values / number of pixels

When Density Data are Displayed in Red

Occasionally, LCGU data may be "flagged" in red. This means that some of the pixels in the sample have density values that do not fall within the range of values established for the [^{14}C] calibration curve. See the Advanced Details section for more information.

Labeling Data

MCID Basic organizes data in a hierarchical fashion as they are gathered:

Subject Animal/subject used in study.

Section Tissue section from subject.

Region Anatomical region within tissue section (e.g., “thalamus”, “cortex-left”).

Target Area of an image sampled under a discrete Region label. A target is outlined or defined by a Sample tool.

Enter the appropriate **Subject, Section** and **Region** labels as you gather data. “Targets” are numbered automatically.

TO CREATE LABELS:

1. Click on one of the **Subject, Section** or **Region** headings. A label dialog box appears on the screen. Each label type has a specific dialog box.
2. Enter the **Subject** (optional), **Section** (optional) and **Region** label in the entry field of the relevant dialog box.
3. Sample the region corresponding to this label.
4. Enter a new **Region** label and continue until you have all read regions in the section.
5. Repeat steps 2 - 4, updating the subject and section labels as required.
6. Click on the **[Done]** buttons to close the dialog boxes.

Figure 11: *The basic set of labels for managing data from sectioned specimens.*

For more information, see *Chapter 10: Data Management* in the online *Reference Manual*.

Step 6: Sampling Remaining Animals/Films

Re-calibration is required whenever you gather data from another animal and/or another film. Once you have established a new LCGU calibration, **MCID Basic** will convert the density value of each pixel that you subsequently sample to a value that corresponds to the new calibration. Data gathered under previous LCGU calibrations are unaffected.

Other Animals

When you are ready to continue the LCGU analysis with another experimental animal (i.e., with another accessory file), go to the **Calibration** operation window and press the **[Re-calibrate]** button. Load the appropriate accessory file and **MCID Basic** will automatically generate a new LCGU calibration curve. It is not necessary to establish a new density calibration curve (ROD vs. nCi/g) unless the sections are on a different sheet of film (see below).

Other Film

A set of concentration standards should ideally be present on every sheet of film used in the study. To establish a new density calibration (i.e., ROD vs. nCi/g), go to the **Calibration** operation window and update the **Value Read** values in the calibration table accordingly. To update these values, place the cursor in the first **Value Read** field and repeat the procedure described in Sampling the Standards, above. As each standard is read, **MCID Basic** also updates the LCGU calibration curve accordingly. If necessary, select a new curve fitting function as well.

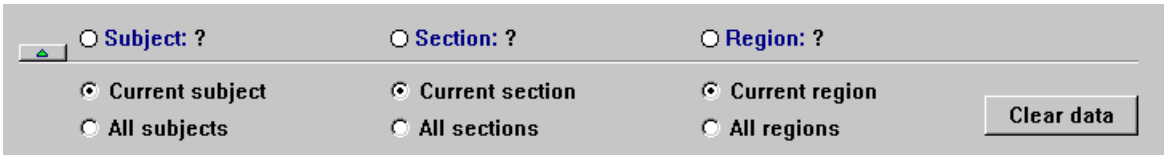
Step 7: Summarizing and Saving Data

MCID Basic contains a number of sophisticated data tracking, reporting and management facilities. If you label your targets as you gather data, you can create a variety of different data display formats that allow you to summarize data across and/or within Subjects, Sections and/or Regions. The **MCID Basic** imaging system's data tracking capabilities can even show you where a given number comes from in an image. You can save data to disk as a proprietary, binary "log" file (*.lg2), or as standard text or spreadsheet files.

It is beyond the scope of this *Application Guide* to present all **MCID Basic** data management features. A few particularly useful summary configuration and saving options are presented below, however. For a complete description, see the online *Reference Manual (see Chapter 10: Data Management)*.

Summary Data Displays

Two sets of controls are used to create data summaries. The first is a set of radio buttons underneath the **Subject**, **Section** and **Region** label indicators (press the  button if they are not visible):



The second is a list of four data Summary Tables located in the **Table** toolbar (*View* > *Table bar*):

All Targets

The **Target Table** is the most detailed form of data display, as it shows a discrete line of data for each individual target. By default, the **MCID Basic** data table lists only the data from the **Current Subject**, **Current Section**, and **Current Region**. To view all of the data you have gathered in one table, change the radio buttons to **All Subjects**, **All Sections**, **All Regions**. Every sample is subsequently displayed by its **Subject**, **Section**, **Region** and **Target** label (Figure 8).

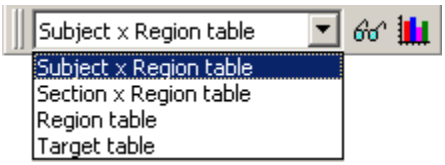
Figure 12: *Part of a Target Table display showing data gathered from All Subjects, All Sections and All Regions.*

Subject	Section	Region	Target	Dens - umol/100g/min
SUB1	SEC2	REG1	1	32.65
			2	42.08
		REG2	1	180.19
			2	196.11
		REG3	1	126.16
			2	132.84
	SEC3	REG1	1	37.45
			2	43.91
		REG2	1	165.54
			2	196.42
		REG3	1	37.25
			2	42.73

Subject x Region Summary Table

Autoradiography data is frequently exported into statistics packages for analysis by Analysis of Variance. The preferred summary format is consequently a table that displays data from each individual subject by each individual anatomical region.

You can display data in tabular format, with **Subjects** as rows, **Regions** as columns. To see the **Subject x Region** view, select the **Subject x Region** summary table:



To pool data across all sections, select the **All sections: Summary** radio button. To display data within each section, select **All sections: Detail**.

Please note that **MCID Basic** can only display one measure at a time in this summary format. **MCID Basic** displays the current measure above the **Subject** label indicator on the left side of the data table. If you have made more than one type of measurement, double-click in this field to scroll to another measure (see Figure 13).

Figure 13: *A Subject x Region data summary. Notice the Subject labels arranged in rows, Regions labels as columns. MCID Basic can display one measure at time in this format.*

Dens - nCi/g	Area1	Area2	Area3	Area4	Area5
rat1	426.13	228.83	33.99	309.71	191.91
rat2	472.98	219.63	32.64	324.83	142.13
rat3	459.74	244.92	39.07	313.11	267.16

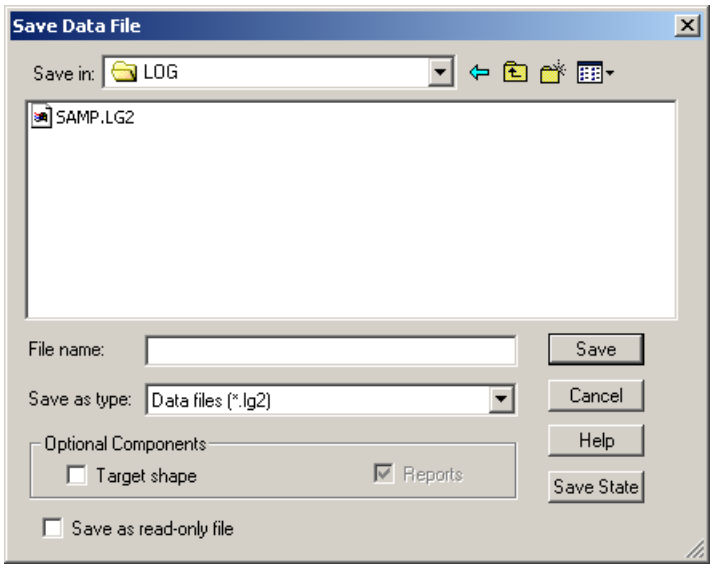
Saving Data

MCID Basic stores data in a proprietary, binary “log” file format (*.lg2). Log files contain more than just numerical data. They contain data, label lists, channel tracking information, and the calibration that was in effect when the file was saved. You can also save target outlines in log files.

TO CREATE A LOG FILE:

1. Go to the *Sample* window and open the *File* menu.
2. Select the *Save as* option. The *Save Data File* dialog box appears (Figure 14).
3. Type a filename into the entry field (e.g., Experiment 1). There is no need to type a file extension; **MCID Basic** always attaches the appropriate extension whenever it saves a file.
4. Press the **[Save]** button. **MCID Basic** stores the file to disk under the specified filename.

Figure 14: *The Save Data File dialog box.*



Re-Opening a Log File

When you open a log file (*File* > *Open*), **MCID Basic** loads its data, labels (*Subject, Section, Region*), and the last density calibration. This is almost like going back to the analysis session when you created the file. You can edit data within the log file, create different data summary configurations, and add new data if you wish.

> **Note:** The calibration contained in the log file is the one that was in effect when you last saved the log file. **MCID Basic** saves calibrated data, but only the most recent calibration. If you intend to continue sampling, we recommend you establish new calibrations.

Saving Target Outlines

To save target outlines with the log file, check the **Target shape** option in the *Save Data File* dialog box. With target outlines saved, you can click on any numerical value and see the target outline from which it came.

Please note that target outlines are linked to channel coordinates, not to specific images. For this feature to be useful (i.e., to be able to re-display the target outline on the *image* from which it came), you have to be able to display both the sample window and the image associated with each numerical value. Images should consequently be saved to disk after they

have been sampled. We recommend using the Subject and Section label entries as image filenames for easy indexing. You want to be able to identify the image associated with each numerical data point.

To see the origin of a data point, retrieve the log file that contains it. Now load the image file that contains the data point that you wish to check. When you click on the data point, the sample window saved with it appears on the image.

Exporting Data

The **MCID Basic** log file format (*.lg2) cannot be read by other programs. To format data for use with other software (e.g., word processing, spreadsheet, database programs), select the *File* > *Export* command. A variety of text file formats are available, including tab delimited (*.txt), comma delimited (*.csv) and space delimited (*.prn). **Lotus® 1-2-3** (*.wks) and **Microsoft® Excel Workbook** (*.xls) files can also be created. Please note, to create *xls files from your data, Excel must be installed on the **MCID Basic** computer.

File > *Export* uses the WYSIWYG (what you see is what you get) principle. Only the data in the current display will be exported, just as it appears on your monitor. You should consequently select all the data display options (e.g., within and across Regions, Sections, Subjects), and the summary options (e.g., **Region** or **Target Summary** table) before exporting your data.

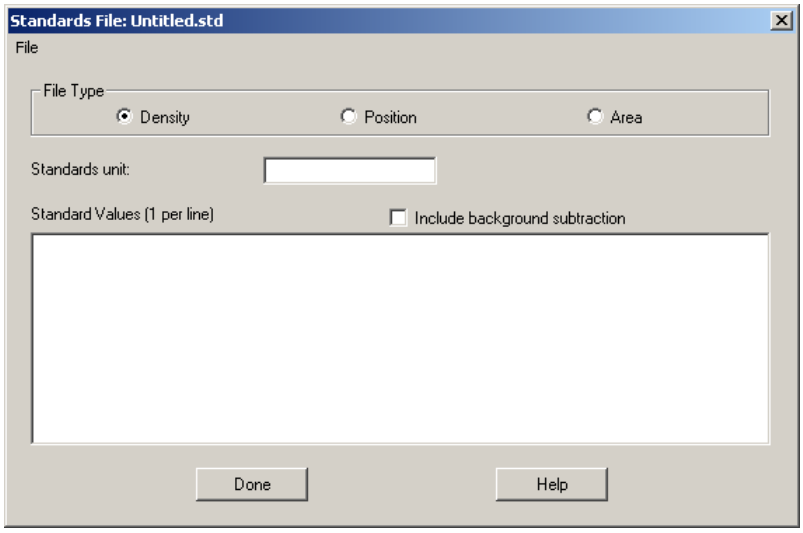
Advanced Details

Creating Standards Files

Most laboratories use a limited number of standard sets as external references. To avoid repetitive typing, you can save the values for each set and retrieve them when you need to calibrate. To create a standards file, either click on the **[Create]** button in the *Open Density Standards File* dialog box (Figure 6) or open the *Calibrations* menu and select *Edit accessory file* > *Calibration standards*. This calls up a dialog box into which you can type the values of your [^{14}C] standards, one per line (Figure 15). Enter the standards unit (e.g., fmol/mg) in the **Standards unit** entry field. Then open the *File* menu in the dialog box and save the file. The file is saved with an *.std extension.

> **Note:** This dialog box has no scroll bars. Use the arrow keys to navigate up and down the list of standard values.

Figure 15: *Creating a file containing the values of external density standards.*



Background Subtraction

For **Density** calibrations, you can use an unexposed part of the film or plate area as the background standard. This represents zero concentration, but the density standards also contain this background value and calibration should take care of the background contribution to standard values.

If you prefer, **MCID Basic** can subtract a background value from each standard before calibration. This procedure can reduce the relative error at the lower end of the calibration curve when weighted regression curves are used (i.e., 1/read, $1/read^2$). To use this feature, click on the **Include background subtraction** checkbox.

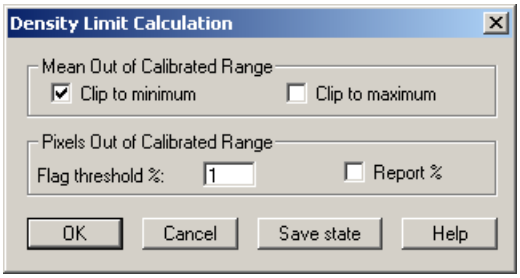
If **Include background subtraction** is enabled, a **Bkgd** term is added to the list of standard values when you open the standards file. Sample an unexposed part of the film as the **Bkgd** standard. When the calibration curve is constructed, this background density value is subtracted from the **Value Read** for each standard. The resulting values are used to derive the parameters used for **1/read** and $\mathbf{1/read^2}$ weighted regression curve types.

When Density Data are Displayed in Red

Occasionally, calibrated density data may be "flagged" in red. This means that some of the pixels in the sample have density values that do not fall within the range of values established for the calibration curve. ***Treat such data with caution***. Your decision to accept or reject the data should depend on a) how many pixels are out of range, b) how far out of range they are, and c) what you've told **MCID Basic** to do with pixels that are out of range.

To find out how many pixels are out of range, open the *Sample* menu and select *Advanced* > *Density limits*. The *Density Limit Calculation* dialog box controls the display of data that is out of calibrated range. Select the **Report %** checkbox and **MCID Basic** will report the proportion of out-of-range pixels in the sample. The proportion is displayed in the data table, next to the flagged data.

Figure 16: *The Density Limit Calculation dialog box controls the display of data from samples that contain pixels that are out of range. Such data is displayed in red.*



By default, **MCID Basic** will flag data when 1% or more of its pixels are out of calibrated range. If you are willing to accept a higher percentage of out-of-range pixels in your samples, enter a higher **Flag threshold**. For example, if you are willing to accept data from a sample with less than ten percent of its pixels out of range, enter a "10" in the **Flag threshold** entry field. **MCID Basic** will then only flag data when 10% or more its pixels are out of range.

By default, **MCID Basic** extrapolates calibrated values for pixels that are out of range. A variety of extrapolation functions are available, and they can be applied to either end of the calibration curve. Alternatively, **MCID Basic** can "clip" out-of-range pixels to the maximum or minimum standard value. For access to extrapolation options, go to the *Calibration* window and press the **[Extrapolate]** button displayed beneath the density calibration curve.

Regardless of whether you choose to extrapolate or clip pixels that are out of range, be advised that these operations are meant to correct for minor excursions beyond the calibration curve. They should not be used as a correction for poor exposures.

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