



Version 6.0

# Application Guide: Receptor Binding

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# Receptor Binding

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## Introduction

Many neurochemicals exert their effects by binding to highly specific receptor proteins. Receptor binding can be localized and quantified by exposing tissue to radiolabeled ligands in the presence and absence of excess unlabeled competitor. The amount of label measured in the *absence* of the unlabeled competitor is taken to represent the “total” binding of the ligand (i.e., that which is bound to specific receptors as well as to other, nonspecific cellular components). The amount of label measured in the *presence* of the competitor is taken to represent the “nonspecific” binding of the ligand. “Specific” binding is then derived by subtracting nonspecific binding from the total.

In autoradiographic experiments, specific binding can be quantified in two ways. The first way is to create a specific binding image. This can be achieved by aligning a nonspecific binding image with a total binding image (preferably serially adjacent sections), and then subtracting the former image from the latter. Quantification is then achieved by sampling the specific binding image.

The second way to measure specific binding in autoradiographic experiments is to sample the total and nonspecific images independently, and then subtract the nonspecific data from the total binding data. This second method tends to yield more accurate data than sampling from a specific binding image, particularly at the extremes of calibration. The image subtraction process is subject to round-off errors, which have less effect on values calculated from discrete total and nonspecific binding images.

The **Receptor Binding** study type utilizes the **AIS™** multi-channel display format and channel-linking features to calculate specific binding values from discrete total and nonspecific binding specimens. Total and nonspecific specimens are first aligned and loaded into separate channels. Sample the total binding image and **AIS** automatically samples the nonspecific image at the same time. The following measures are then reported in the data table:

**Tot Bind** -- Value from the total binding image.

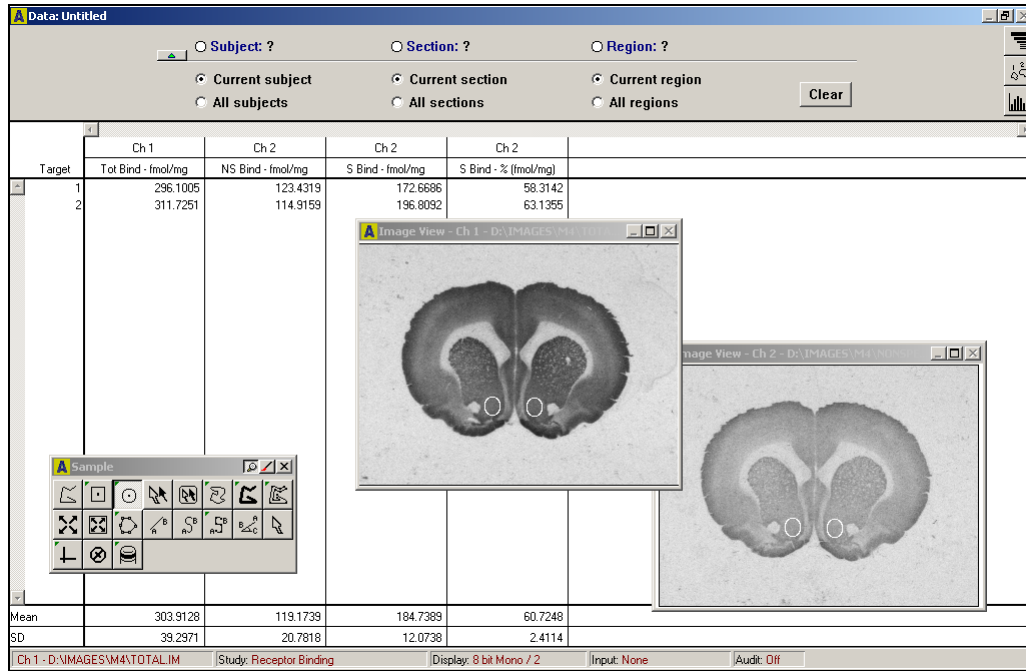
**NS Bind** -- Value from the nonspecific binding image.

**S Bind** -- Specific binding (total binding value minus nonspecific binding value).

**S Bind-%** -- Specific binding, expressed as a proportion of total binding value.

If alignment of a nonspecific image to a total binding image is neither necessary nor feasible, the “nonspecific” channel can be set to a single predetermined value.

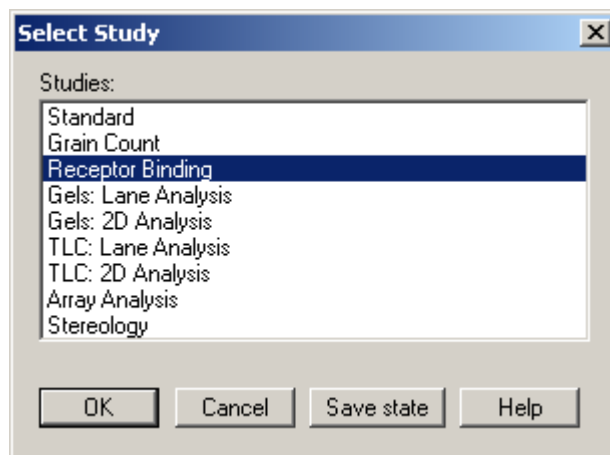
**Figure 1:** The Sample window for the “Receptor Binding” study type. Total and nonspecific binding images are aligned and placed into Channels 1 and 2 respectively. Sample the total binding image and AIS automatically samples the same region of interest in the nonspecific binding image. Total, nonspecific and specific binding values are reported in the data table.



## Basics

To enable the **Receptor Binding** study type, select *Study > Select > Receptor Binding*.

**Figure 2:** Enabling the Receptor Binding study type.



The **Receptor Binding** study type requires a multi-channel **Display Format** (i.e., at least two channels). Before beginning, open the *Settings* menu and select one of the multi-channel display types.

The basic procedure for quantifying levels of specific binding is as follows:

1. Digitize a total binding image into Channel 1.
2. Use one of the methods below for assigning nonspecific binding to Channel 2.
3. Using any sample tool, define a region of interest in the total binding image (Channel 1) and sample it. **AIS** automatically samples the same region of interest in Channel 2 (nonspecific binding). Total, nonspecific and specific binding values for the region of interest are reported in the data table.
4. Repeat step 3 for all other regions of interest in the total binding image. Change Region labels as required.
5. Repeat steps 1-4 for all other total binding images. Change Subject and Section labels as required.

## Assigning Nonspecific Binding Values

### Aligning Nonspecific Binding Specimens

The most accurate way to derive specific binding values from total binding specimens is to subtract the amount of nonspecific binding present in adjacent sections. Since **AIS** is configured to sample the same region of interest in both images at once, the nonspecific binding specimen must be in alignment with the total binding specimen.

#### TO ALIGN A NONSPECIFIC SPECIMEN WITH A TOTAL BINDING SPECIMEN:

1. Digitize the total binding specimen into Channel 1.
2. Click the **Alignment** icon:



3. Two shadowy images are now visible, one on top of the other. One is the total binding specimen, which is “frozen” in place. The other is a live image, which is actively digitizing. The live image may be moved (by hand) into alignment with the stored image.
4. Move the nonspecific binding specimen into the field of view and position it until it lies directly over the total binding specimen.
5. Press the **[OK]** button. The total binding image remains in Channel 1, and the aligned nonspecific binding specimen is automatically placed into Channel 2.

**Note:** When working with multiple images in multiple channels, make certain that each channel contains the correct calibration. Total and nonspecific binding images need not share the same calibration (i.e., they do not have to come from the same sheet of film), but they must be calibrated to the same units.

### Using a Single, Predetermined Value

It is not always necessary (or feasible) to have an adjacent nonspecific binding specimen to align with each total binding specimen. Many ligands, for example, exhibit little or no anatomical variation in nonspecific binding. In *in vivo* experiments, nonspecific and total binding specimens will necessarily come from different animals, and perfect anatomical alignment with sections from different animals may be difficult to achieve. In cases such as these, the nonspecific binding channel (Channel 2) may be set to a predetermined density value. This value is set by i) typing in a value or ii) sampling from an area or specimen that represents nonspecific binding.

#### TO ENTER A PREDETERMINED NONSPECIFIC BINDING VALUE:

1. Open the *Receptor* menu and select *Set nonspecific binding*. A *Set Nonspecific Binding* dialog box is displayed.
2. Type the desired value in the entry field, then click **[OK]**. Channel 2 will be set to a shade of gray that corresponds to the specified density value.

#### TO SET A NONSPECIFIC BINDING VALUE BY SAMPLING:

1. Digitize a nonspecific binding specimen into Channel 1 or Channel 2.
2. Open the *Receptor* menu and select *Set nonspecific binding*.
3. Using any sample tool, sample a region of nonspecific binding. As you sample, the value is displayed in the *Set Nonspecific Binding* dialog box. Click **[OK]** to exit.

Once a nonspecific binding value has been specified, Channel 2 will be set to a shade of gray that corresponds to the specified value. This value will be subtracted from all targets that are *subsequently* sampled from images contained Channel 1.

## Advanced Details

### Combining Receptor Autoradiography and Histology

In some autoradiographs, anatomical regions are difficult to define. One way to assist the definition process is to image a counter-stained section from which the autoradiograph was made, and align it with the autoradiographic image. With the AIS imaging system's channel-linking feature, the counter-stain image may then be used to define anatomical regions, while data is gathered from within the aligned autorad.

Selecting the **Receptor Binding** study type automatically links Channel 1 (total binding) and Channel 2 (nonspecific binding). To use a counter-stain image to define regions of interest in the total binding image --

1. Open the *Settings* menu and select a 4-channel *Display Format*.
2. Digitize the counter-stained total binding specimen into Channel 4.

3. Press the **Alignment** icon and align the corresponding autoradiographic specimen with the counter-stain image (see above for alignment instructions). The autoradiographic image will automatically be placed into Channel 1.
4. Use one of the methods described above for assigning nonspecific binding to Channel 2.
5. Using any **Sample** tool, define a region of interest in the counter-stain image. **AIS** automatically gathers data from the same region of interest in Channel 1 (the total binding image) and Channel 2 (nonspecific binding image). Total, nonspecific and specific binding values for the region of interest are reported in the data table.

### Creating a Specific Binding Image

A “Specific Binding” image may be created by i) subtracting a nonspecific binding image from a total binding image, or ii) subtracting a single predetermined nonspecific binding value from the total binding image. Although we do not recommend gathering data from such images (see above), they are useful for localizing regions of specific binding in visual presentations.

#### Subtracting Images

To subtract a nonspecific binding image from a total binding image --

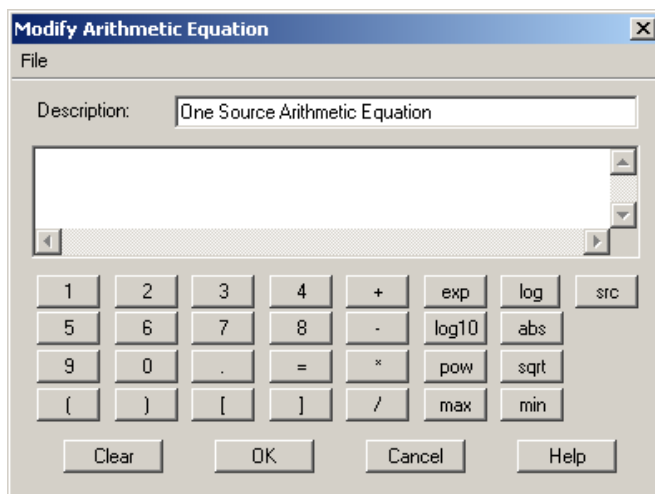
1. Digitize the total binding autoradiograph into Channel 1.
2. Press the **Alignment** icon and align the corresponding nonspecific binding specimen with the total binding specimen (see above for alignment instructions). The aligned image will automatically be placed into Channel 2.
3. Open the **Transform** operation window.
4. Select “#Subtraction” from the list of **Point Operators**.
5. Specify Channel 1 as the first source channel (i.e., enter “1” in the “Source1” entry field).
6. Specify Channel 2 as the second source channel (i.e., enter “2” in the “Source2” entry field).
7. Specify Channel 3 as the destination channel (i.e., enter “3” in the “Destination” entry field).
8. Press the **[Apply]** button. **AIS** will subtract Source2 (nonspecific binding) from Source1 (total binding) and place the result (specific binding) in Channel 3. If calibrations are in effect then the calibration from Source1 will be applied to the resultant image.
9. Save the image in Channel 3 under a new filename.

### Subtracting a Predetermined Value

To subtract a single nonspecific binding value from a total binding image --

1. Digitize a total binding autoradiograph into Channel 1
2. Go to the **Calibration** window and open the *Establish* menu. Select the *Copy calibration* option and copy the calibration from Channel 1 to Channel 2.
3. Go to the **Transform** operation window and select “#Arithmetic (One Src)” from the list of **Point Operators**.
4. Specify Channel 1 as the **Source** channel and Channel 2 as the **Destination** channel.
5. Click on the [**Modify**] button. A *Modify Arithmetic Equation* dialog box appears (Figure 3).
6. Click on the keypad buttons to construct the equation **src - x**, where **x** is the nonspecific binding value, in calibrated units. Click [**OK**] to exit.
7. Click the [**Apply**] button. **AIS** will subtract the nonspecific binding value from the Source image (total binding) and place the result (specific binding) in Channel 2.
8. Save the image in Channel 2 under a new filename.

*Figure 3: The Modify Arithmetic Equation dialog box.*



### Aligning Images with 'Image Registration'

If you are working with image files, and if the nonspecific binding images were not aligned with total binding images when the images were acquired, it is still possible to align them by “registration”.

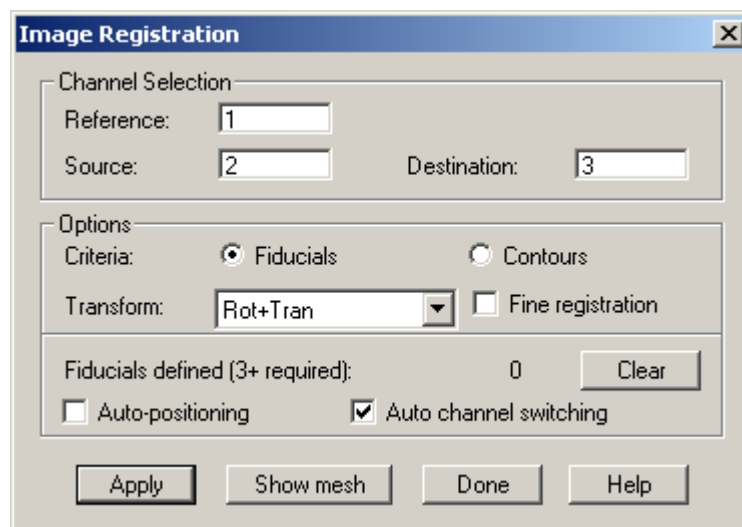
#### TO ALIGN IMAGES BY REGISTRATION:

1. Load a total binding image into Channel 1, and the non-aligned nonspecific binding image into Channel 2.

2. Open the **Transform** operation window and select *Image Registration* from the *Transform* menu. An *Image Registration* dialog box is displayed (Figure 4).
3. Specify the “Reference”, “Source” and “Destination” channels. In this example, we will align the nonspecific image (Channel 2) with the total binding image (Channel 1) and place the result in Channel 3. Enter “1” for the reference channel, “2” for the source channel, and “3” as the destination channel.
4. Alignment will be achieved by rotating and shifting (i.e., translating) the nonspecific image until a specified set of anatomical landmarks (fiducial points) match the total binding image. Select “Rotat+Trans” as the Transform function and select “Fiducials” as the alignment criteria.
5. Move to the reference channel (Channel 1) and define a set of fiducial points. To define a fiducial point, position the cross-hair cursor over an anatomical landmark and click. As each fiducial point is defined, a matching point appears in the same XY position in the source channel (Channel 2). Define at least four fiducial points.
6. Move to the source channel (Channel 2) and position the cursor near one of the matching fiducial points. When the cursor touches a marker, it turns into an arrow. Hold down the left mouse button and drag the marker to its correct *anatomical* position (i.e., to the same anatomical landmark used in the reference image).
7. Repeat Step # 6 for the remaining fiducial points, then click the [**Apply**] button.

The selected transformation (Rotation+Translation) is then used to spatially align the fiducial points on the source image with their matching points on the reference image. The aligned image is placed into the designated destination channel (Channel 3). Click [**Done**] when the transformation is complete, and save the image in Channel 3 under a new filename. When using the **Receptor Binding** study type, load the aligned image into Channel 2, and the corresponding total binding image into Channel 1.

**Figure 4:** The *Image Registration* dialog box.



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**InterFocus Imaging Ltd  
Cambridge Road  
Linton  
CB1 6NN  
England**

**TEL: +44 (0)1223 894833 – FAX: +44 (0)1223 894235**

**US & Canada Toll Free: 1-866-782-2202 – Fax: 1-917-591-9130**

**Email: [sales@mcid.co.uk](mailto:sales@mcid.co.uk)**

**Visit MCID Online at [www.mcid.co.uk](http://www.mcid.co.uk)**