Illumination Reference Files for the In-Vivo & IS4000 Imaging Systems

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Theory

To accurately measure multiple samples in a single image it is necessary to correct for unavoidable variations in the uniformity of the excitation illumination field across the field of view of the image.

Variations across the imaging field of view arise from:

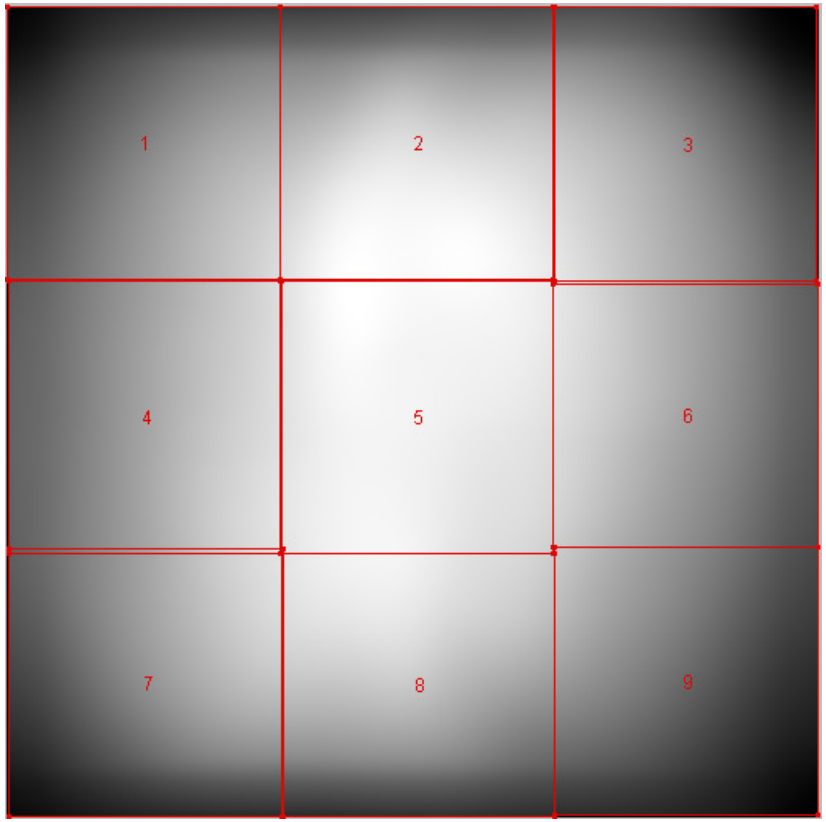
- Excitation Nonuniformity
- Detection Nonuniformity

When do we need Illumination Reference files?

- For High Resolution X-ray Images
  - Corrects for Detector Nonuniformity (CCD & confocal lens)
- To make comparisons between fluorescent samples across a field of view
  - Corrects for Detector & Excitation Nonuniformities
- Illumination reference files are specific to: Illumination source (type of capture), X-ray filter (X-ray only), f-stop, FOV, Focal Plane, Excitation filter, Emission Filter. If one of these parameters is altered, a new illumination reference must be taken
- Illumination reference files are not specific to exposure time or binning.

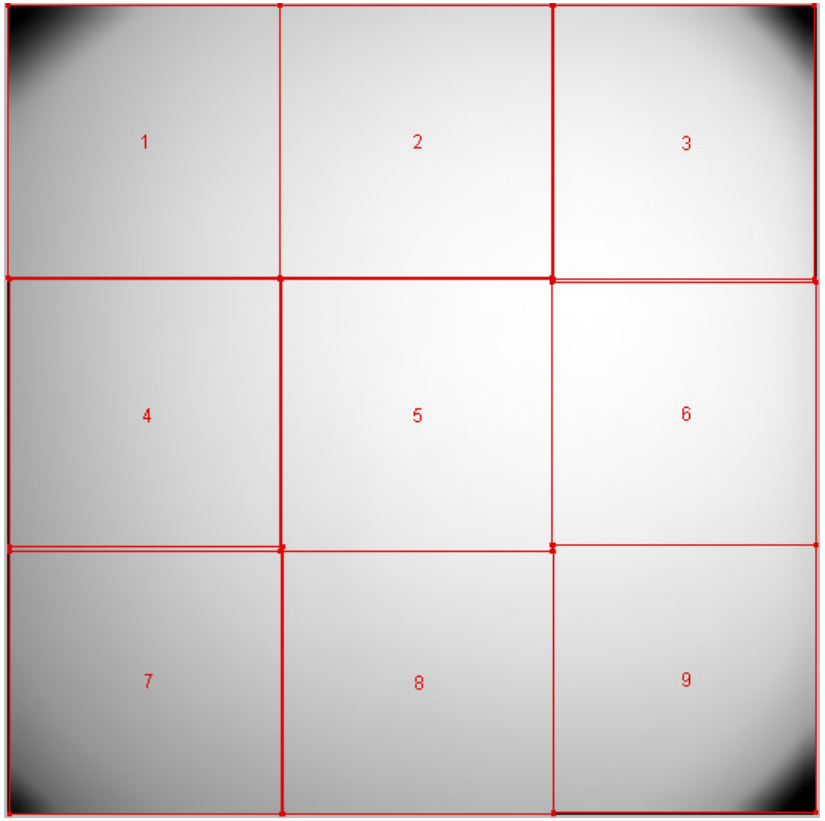
Nonuniformity Detailed

Fluorescence Illumination Reference File



1	2	3	4	5	6	7	8	9
2210	3500	2373	2983	4280	2921	2358	3150	1852

X-ray Illumination Reference File

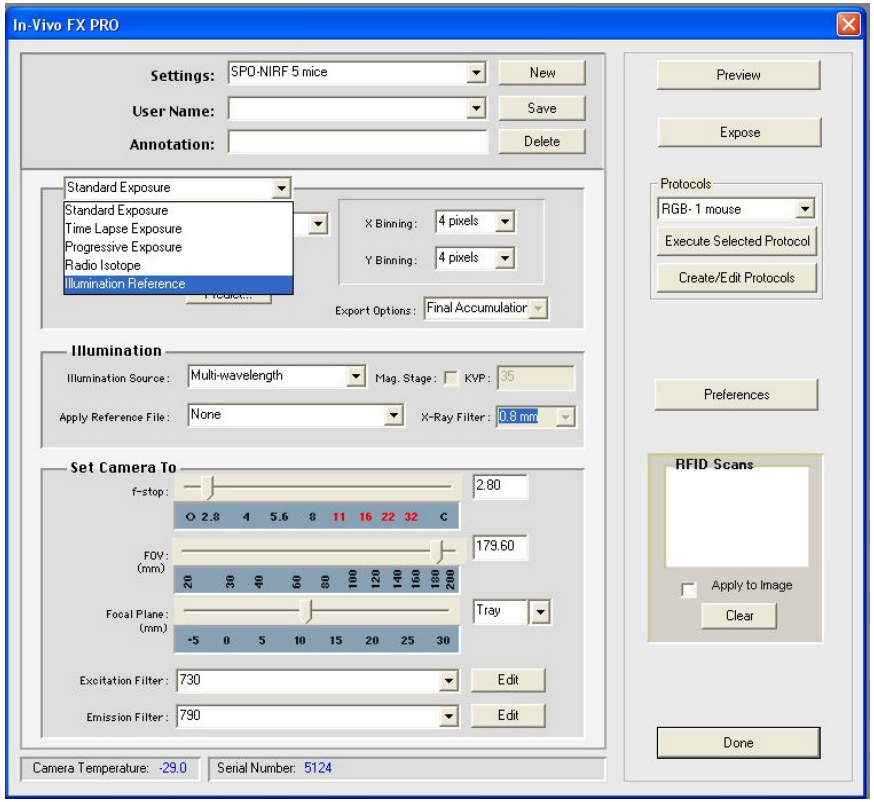


1	2	3	4	5	6	7	8	9
15236	18854	18258	16293	19407	19502	13711	16598	16153

Significant differences seen in the mean intensity per image region in both fluorescence & X-ray captures…it is this nonuniformity that must be corrected for quantitative/qualitative comparisons!!!

How to Make an Illumination Reference: Fluorescence

1) Establish your fluorescent capture settings and then select illumination reference in the exposure type:



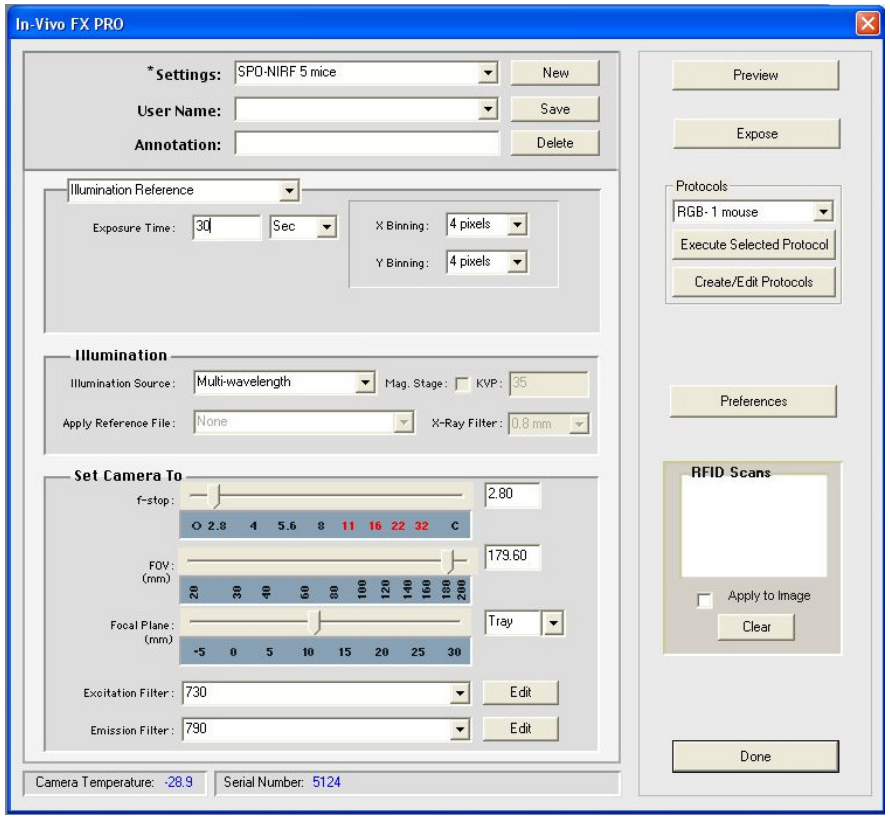
2) Place your Epi Field Flattening screen into your system covering the platen completely

How to Make an Illumination Reference: Fluorescence (cont'd)

3) Select Exposure time and binning.

Note: Binning will always be 4x4 unless 8x8 binning is used in capture setting. Exposure time needed varies by emission filter due to efficiency differences of the epi field flattening screen

175W		400W	
Em Filter	**Exp time(s)**	**Em Filter**	**Exp time(s)**
480	0.3	480	0.2
535	1	535	0.5
600	1.5	600	1
670	3	670	2
700	5	700	3
750	10	750	5
790	60	790	30
830	180	830	60



4) Press Expose Button

5) Do not "save" capture setting file

How to apply an Illumination Reference File: Fluorescence

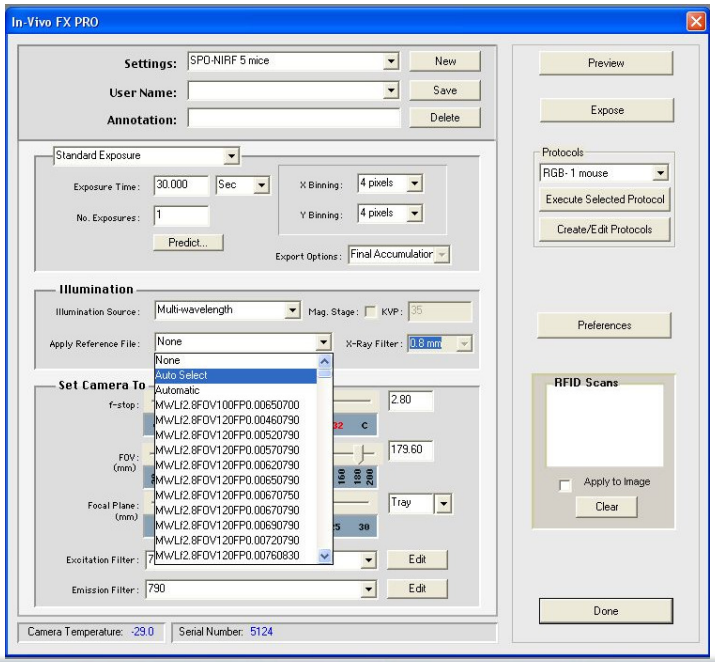
Automatic Application to future captures

After you have successfully captured your illumination reference file:

Select Apply Reference file -> Auto Select

If the reference file is currently open, it will disappear from the screen, indicating a proper application

Images will automatically be processed with appropriate reference file if no error message occurs

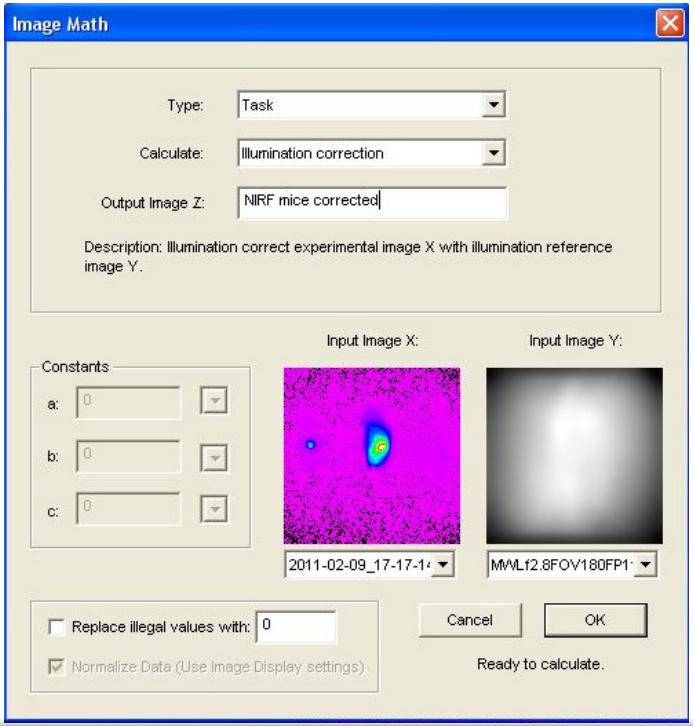


Application to a pre-existing capture

Open image to be corrected and the proper reference file

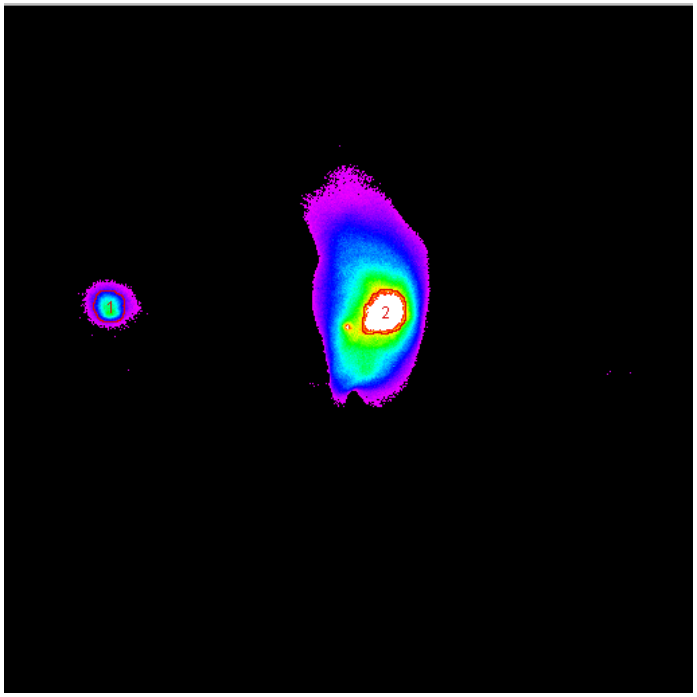
Navigation panel -> Image -> Image Math

Model after below screen shot

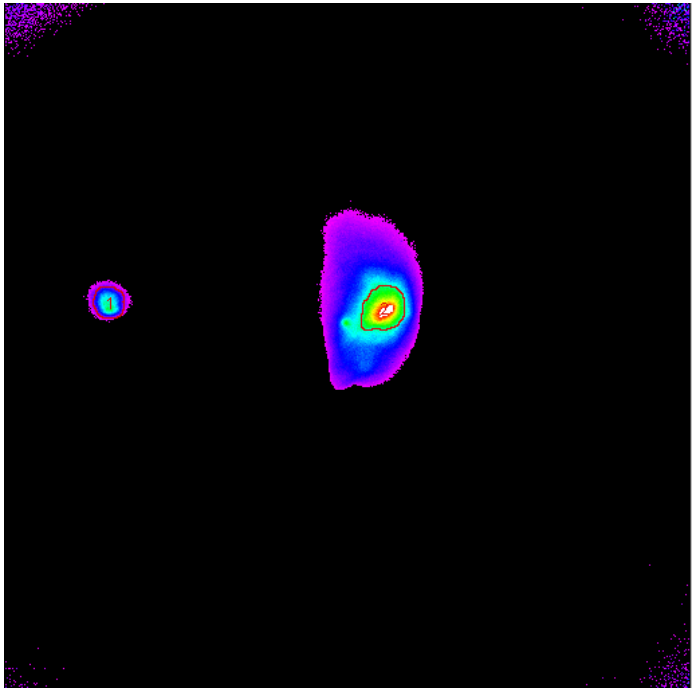


Results of Illumination Reference File Application: Fluorescence

No Reference File Applied



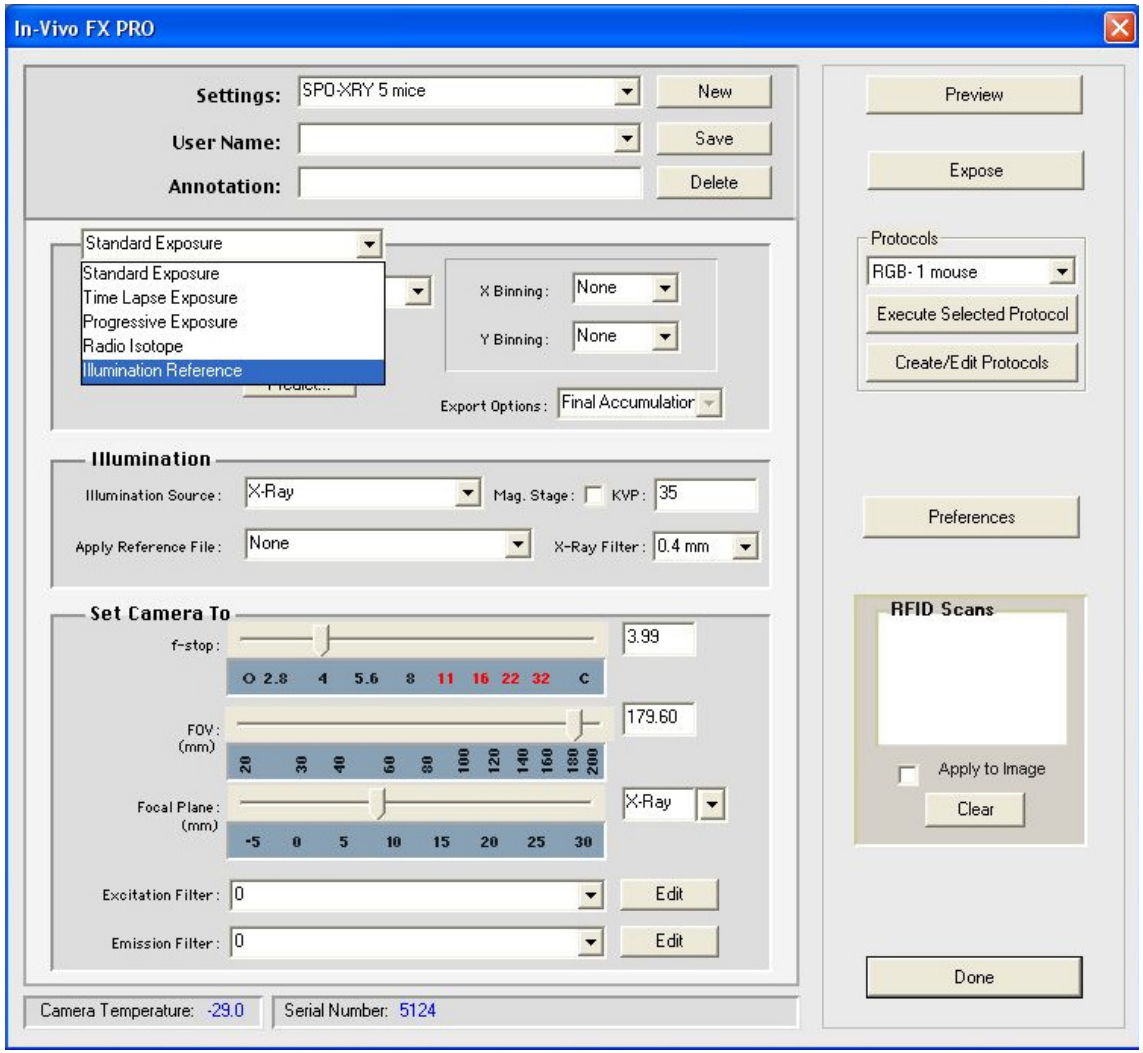
With Reference File Applied



Mean Intensity ROI 1 No Reference	Mean Intensity ROI 1 With Reference	Percent Difference	Mean Intensity ROI 2 No Reference	Mean Intensity ROI 2 With Reference	Percent Difference
1205	1092	9.38 %	3529	2217	37.18%

Significant Signal Quantification error can occur without illumination reference

How to Make an Illumination Reference: X-ray



1) Create desired captured setting
2) Select Illumination Reference
3) Select Reference File Settings. Always 4x4 binning. Exposure time varies depending on X-ray filter used

None = 10s exposure
0.1 mm = 15s exposure
0.2 mm = 20s exposure
0.4 mm = 30s exposure
0.8 mm = 30s exposure

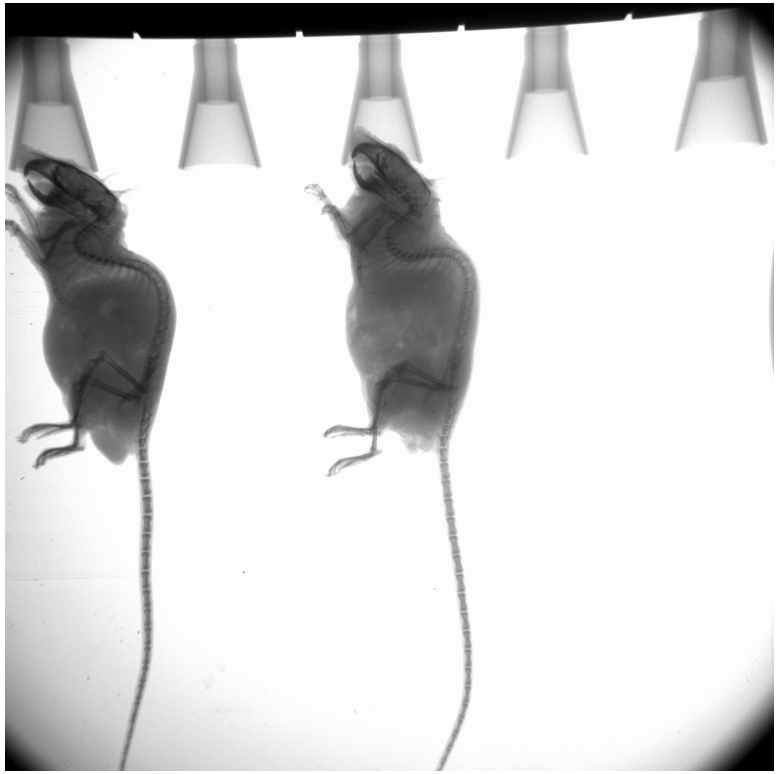
4) Remove all samples from image station
5) Expose
6) Do not “save” to capture setting file

How to apply an Illumination Reference File: X-ray

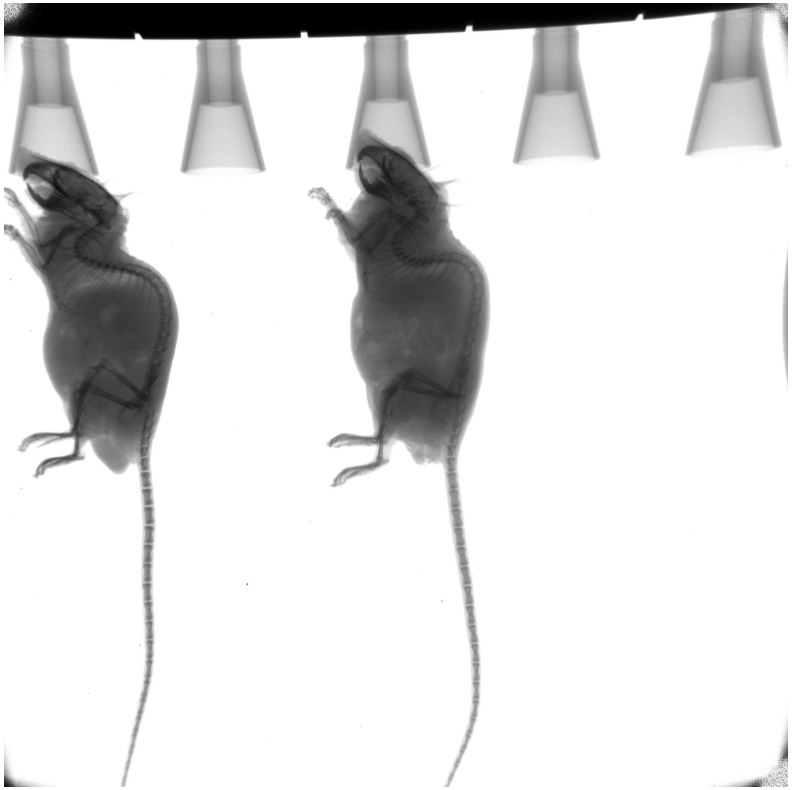
Refer to “How to apply an Illumination Reference File: Fluorescence” slide. It is the same!!!

Results of Illumination Reference File Application: X-ray

No Reference File Applied



With Reference File Applied



- Reference file application smoothes image and removes low-signal corners
- Reference files essential for X-ray density conversion and bone density analysis

Thank You!!