

Falcon Individual Frame Read Out Mode User Manual

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There are three files that are required to install the Falcon individual frame read out mode, FalconInterImageTool.exe, IntermediateConfig.xml, and a text file that contains the read out time for the falcon (Program_Table.txt). The required software version is Titan 1.4/1.5 or Tecnai 4.4/4.5.

The FalconInterImageTool.exe should be copied and placed into the following path C:\Titan\Exe. When activated, the .exe opens a GUI (Figure 1) that contains two experimental options, Normal acquisition mode and store intermediate images. There is also an option to view the intermediate images in TIA button that imports the individual frames into TIA for viewing. The normal acquisition mode saves integrated images and the store intermediate images saves individual frames predetermined by a set of parameters which can be changed in the IntermediateConfig.xml file.

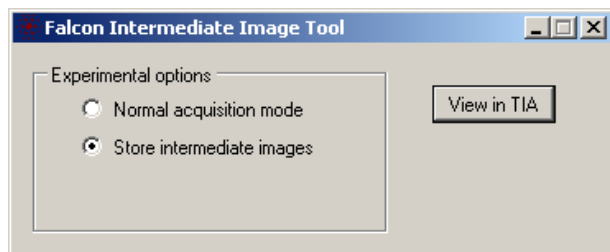


Figure 1. GUI of the intermediate image tool.

The IntermediateConfig.xml file should be opened with notebook.

```
<?xml version="1.0" encoding="utf-8"?>
<IntermediateConfig>
  <FormatVersion>1.0</FormatVersion>
  <StoragePath>F:\single frame\sample_xyz</StoragePath>
  <InterFrameBoundary>0.084 - 0.084</InterFrameBoundary>
  <InterFrameBoundary>0.139 - 0.139</InterFrameBoundary>
  <InterFrameBoundary>0.195 - 0.195</InterFrameBoundary>
  <InterFrameBoundary>0.251 - 0.251</InterFrameBoundary>
  <InterFrameBoundary>0.307 - 0.307</InterFrameBoundary>
  <InterFrameBoundary>0.363 - 0.697</InterFrameBoundary>
  <InterFrameBoundary>0.418 - 0.418</InterFrameBoundary>
</IntermediateConfig>
```

Figure 2. An example of an IntermediateConfig.xml file that shows a predetermined set of parameters in which the seven raw images will be fully illuminated individual frames. It also shows the storage location of the raw images - F:\single frame\sample_xyz.

Situation SW Titan 1.4/Tecnai 4.4

First, it needs to be determined if the user Falcon saves an INI file or not. This can be seen if you open the logviewer under C:\Titan\log\Imaging\Falcon.log. Scroll up to find a start acquisition message. Four lines below this is the exposure time and frame time, an example is 55.771ms per frame. This means that each frame acquired takes 55.771ms.

Situation SW Titan 1.5/ Tecnai 4.5

From SW Titan 1.5 /Tecnai 4.5, by default the frame time is set to 55.771ms.

Up to 7 dose fractions can be programmed. A fraction can be one frame or several consecutive frames added together. Each fraction is defined by the midpoints of the first frame and the last frame. An example of 7 dose fractions is shown in Figure 2.

Some notes on the setting parameter programming:

- The Falcon reads out by using a rolling shutter. That means that the first frame `<InterFrameBoundary>0.028 - 0.028</InterFrameBoundary>` will display a gradient of accumulated intensity. Similarly, the last two frames also show a gradient.
- Dose fractions should not extend beyond the total exposure time including the run-in and run-out of the rolling shutter.
- Dose fractions are not allowed to overlap.
- Error handling: In case of an error related to the intermediate image mode the camera goes offline. In C:\Titan\log\Imaging\Falcon.log the rootcause error message can be found. You can restore the camera by pushing the button “camera to online”. Most likely, the file path is incorrect, dose fractions overlap, or the exposure time is shorter than the programmed time span of the dose fractions.

There are two ways to transfer the gain reference to the individual frame read out mode. First after the acquisition mode is changed to ‘Store intermediate images’ acquire a new gain reference. The gain reference images are saved into the mode7 folder in C:\Titan\Data\falcon. Alternatively, make a new folder called mode7, copy all the images and files from mode4 (situation SW Titan 1.4/Tecnai 4.4) or mode8 (situation SW Titan 1.5/Tecnai 4.5) to the mode7 folder.

The individual images are stored in the FEI raw image format.

FEI raw image format

offset	size	content
0	13	"FEI RawImage"
13	4	1
17	4	width
21	4	height
25	4	channels (1)
29	4	bits
33	4	encoding
37	4	offset
41	4	stride_x
45	4	stride_y
49	N	data

Encoding

1	RawImage_Signed
---	-----------------

N = Width x Height

area	binning		
	1	2	4
quarter	1024 ²	512 ²	256 ²
halve	2048 ²	1024 ²	512 ²
full	4096 ²	2048 ²	1024 ²

Figure 3. A description of the FEI raw image format.

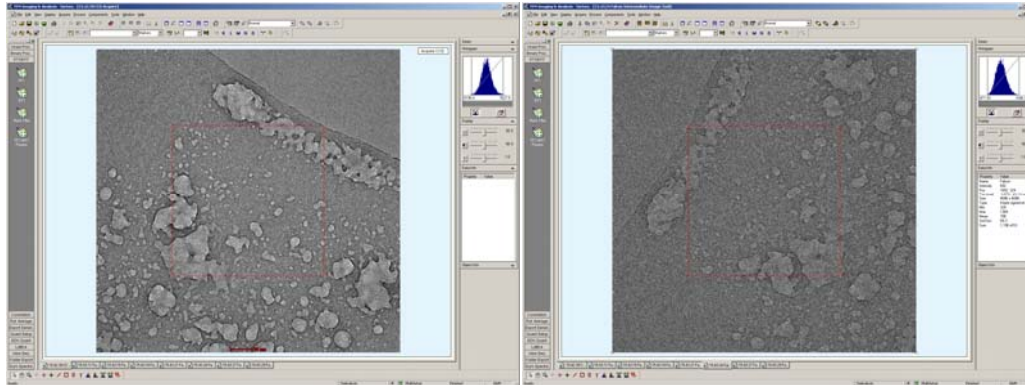


Figure 4. Left is an integrated image and the right is an intermediate image (note the rotation and flip is different from the integrated image).

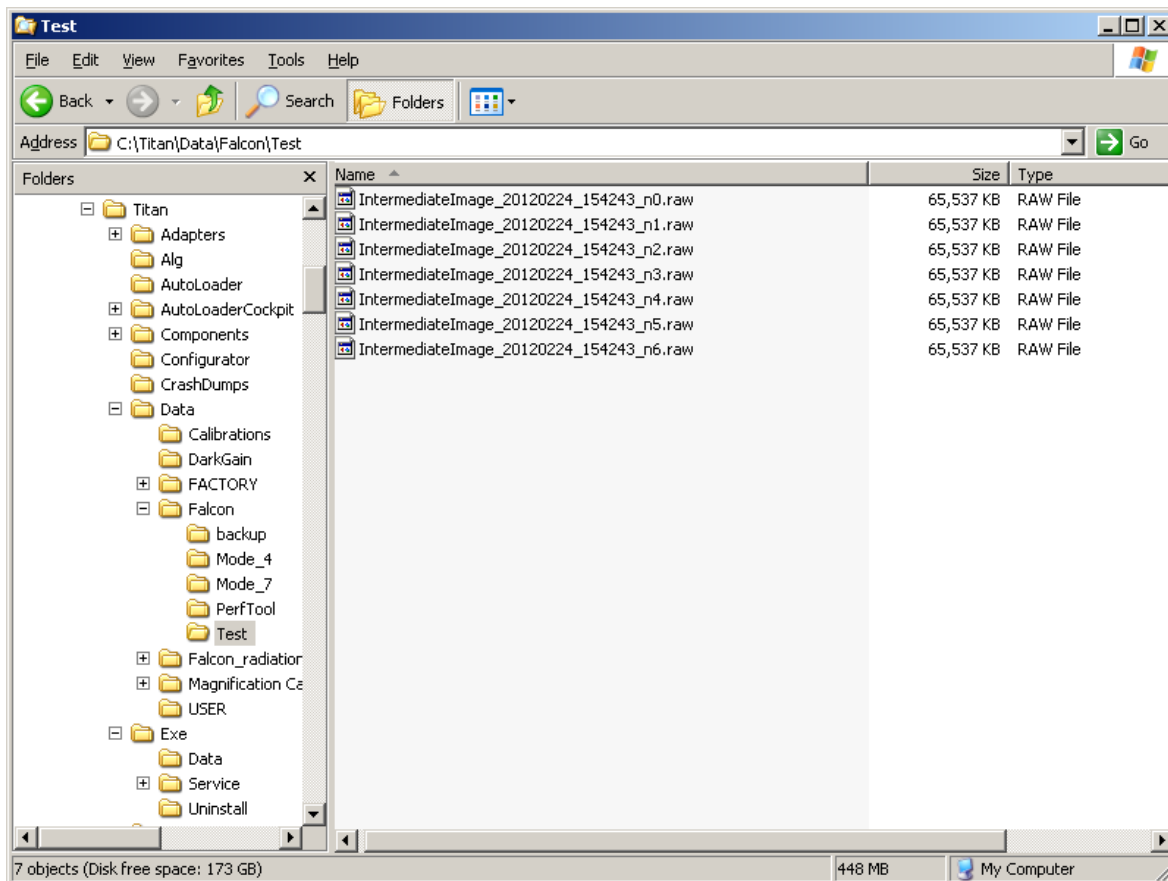


Figure 5. Each file is uniquely numbered by a time and date stamp and a number n0 till n6.