

# Generate a defect file for cryoSPARC

Gain/defect files coming from Glacios must be flipped along Y axis when imported in cryoSPARC.

Gain file is by default named CountRef...mrc (with a pixel size of 1)

Defect file is by default a text file

When importing the movies you should give a defect map file as well as the gain reference file.

Load IMOD

```
module load imod (on cbi-... servers)
setimod (on xtallo machines)
```

You can make a defect map from the text file with 'clip defect' in IMOD 4.10.7 or higher:

```
clip defect -D defects...txt fileWithFrames defects...mrc
```

where the fileWithFrames is used only to set the size of the output and can be any file of the right X and Y size.

If the defect text file is not recognized by cryoSPARC, the Gain file has to be modified to put a value of 0 on defective pixels (because any pixels that have a value of 0 in the gain reference file are treated as defects).

In the Gain file, the defective pixels have a value of 1, so you need to subtract the defects...mrc file from the Gain...mrc file

```
subimage Gain...mrc defects...mrc Gain_subimage...mrc
```

The pixel size will be messed-up and has to be fixed to get back to a value of 1

```
alterheader -fixpixel Gain_subimage...mrc
```

Now the Gain\_subtracted...mrc file has a zero value for defective pixels instead of 1.

Remark: the defect file generated by the Glacios now (2021-10-27) is a map mode 2 and has a pixel spacing of 0.9372, but the images have a pixel spacing of 0.901. It might be better to change the last command by

```
alterheader -del 0.901,0.901,0.901 Gain_subimage...mrc
```

```
subimage CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1.mrc
defects_40S_ScVdelta_03939_Oct27_11.34.51.mrc
CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1_subimage.mrc
```

```
R0 image file on unit 2 : defects_40S_ScVdelta_03939_Oct27_11.34.51.mrc
Size= 13906 K
```

```

Number of columns, rows, sections ..... 3838 3710 1
Map mode ..... 2 (32-bit real)
Start cols, rows, sects, grid x,y,z ... 0 0 0 3838 3710
1
Pixel spacing (Angstroms)..... 0.9372 0.9372 0.9372
Cell angles ..... 90.000 90.000 90.000
Fast, medium, slow axes ..... X Y Z
Origin on x,y,z ..... 0.000 0.000 0.000
Minimum density ..... 0.85517E-01
Maximum density ..... 4.5518
Mean density ..... 1.0010
tilt angles (original,current) ..... 0.0 0.0 0.0 0.0 0.0 0.0
Space group,# extra bytes,idtype,lens . 0 0 0 0

```

1 Titles :

Digital Micrograph(TM), GMS v 3.32

```

Number of columns, rows, sections ..... 3838 3710 1
Map mode ..... 0 (byte)
Start cols, rows, sects, grid x,y,z ... 0 0 0 3838 3710
40
Pixel spacing (Angstroms)..... 0.9010 0.9010 0.9010
Cell angles ..... 90.000 90.000 90.000
Fast, medium, slow axes ..... X Y Z
Origin on x,y,z ..... 0.000 0.000 0.000
Minimum density ..... 0.0000
Maximum density ..... 1.0000
Mean density ..... 0.70932E-05
tilt angles (original,current) ..... 0.0 0.0 0.0 0.0 0.0 0.0
Space group,# extra bytes,idtype,lens . 0 0 0 0

```

4 Titles :

```

SerialEMCCD: Dose frac. image, scaled by 1.00 r/f 0
CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1.dm4
defects_40S_ScVdelta_03939_Oct27_11.34.51.txt
clip defectmap: Map of defective pixels in image 28-Oct-21 11:40:50

```

```

NEW image file on unit 3 :
CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1_subimage.mrc
Section      Min      Max      Mean      S.D.
0            0.0000  4.5518  1.0010   0.0327
alterheader -fixpixel
CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1_subimage.mrc

```

```

OLD image file on unit 2 :
CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1_subimage.mrc Size=
55622 K

```

```

Number of columns, rows, sections ..... 3838 3710 1

```

```

Map mode ..... 2 (32-bit real)
Start cols, rows, sects, grid x,y,z ... 0 0 0 3838 3710
1
Pixel spacing (Angstroms)..... 0.9372 0.9372 0.9372
Cell angles ..... 90.000 90.000 90.000
Fast, medium, slow axes ..... X Y Z
Origin on x,y,z ..... 0.000 0.000 0.000
Minimum density ..... 0.0000
Maximum density ..... 4.5518
Mean density ..... 1.0010
tilt angles (original,current) ..... 0.0 0.0 0.0 0.0 0.0 0.0
Space group,# extra bytes,idtype,lens . 0 0 0 0

```

2 Titles :

```

Digital Micrograph(TM), GMS v 3.32
SUBIMAGE: Subtract section B from section A. 28-Oct-21 11:42:19

```

Changing sample and cell sizes to match image size, which will make pixel spacing be 1.0 1.0 1.0.

R0 image file on unit 3 :

```

CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1_subimage.mrc Size=
55622 K

```

```

Number of columns, rows, sections ..... 3838 3710 1
Map mode ..... 2 (32-bit real)
Start cols, rows, sects, grid x,y,z ... 0 0 0 3838 3710
1
Pixel spacing (Angstroms)..... 1.000 1.000 1.000
Cell angles ..... 90.000 90.000 90.000
Fast, medium, slow axes ..... X Y Z
Origin on x,y,z ..... 0.000 0.000 0.000
Minimum density ..... 0.0000
Maximum density ..... 4.5518
Mean density ..... 1.0010
tilt angles (original,current) ..... 0.0 0.0 0.0 0.0 0.0 0.0
Space group,# extra bytes,idtype,lens . 0 0 0 0

```

2 Titles :

```

Digital Micrograph(TM), GMS v 3.32
SUBIMAGE: Subtract section B from section A. 28-Oct-21 11:42:19

```

alterheader -del 0.901,0.901,0.901

```

CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1_subimage.mrc

```

OLD image file on unit 2 :

```

CountRef_9AR3_00001_Oct27_15.55.18_X+0Y+0-1_subimage.mrc Size=
55622 K

```

```

Number of columns, rows, sections ..... 3838 3710 1

```

```

Map mode ..... 2 (32-bit real)
Start cols, rows, sects, grid x,y,z ... 0 0 0 3838 3710
1
Pixel spacing (Angstroms)..... 1.000 1.000 1.000
Cell angles ..... 90.000 90.000 90.000
Fast, medium, slow axes ..... X Y Z
Origin on x,y,z ..... 0.000 0.000 0.000
Minimum density ..... 0.0000
Maximum density ..... 4.5518
Mean density ..... 1.0010
tilt angles (original,current) ..... 0.0 0.0 0.0 0.0 0.0 0.0
Space group,# extra bytes,idtype,lens . 0 0 0 0

```

2 Titles :

Digital Micrograph(TM), GMS v 3.32  
SUBIMAGE: Subtract section B from section A. 28-Oct-21 11:42:19

R0 image file on unit 3 :  
CountRef\_9AR3\_00001\_Oct27\_15.55.18\_X+0Y+0-1\_subimage.mrc Size=  
55622 K

```

Number of columns, rows, sections ..... 3838 3710 1
Map mode ..... 2 (32-bit real)
Start cols, rows, sects, grid x,y,z ... 0 0 0 3838 3710
1
Pixel spacing (Angstroms)..... 0.9010 0.9010 0.9010
Cell angles ..... 90.000 90.000 90.000
Fast, medium, slow axes ..... X Y Z
Origin on x,y,z ..... 0.000 0.000 0.000
Minimum density ..... 0.0000
Maximum density ..... 4.5518
Mean density ..... 1.0010
tilt angles (original,current) ..... 0.0 0.0 0.0 0.0 0.0 0.0
Space group,# extra bytes,idtype,lens . 0 0 0 0

```

2 Titles :

Digital Micrograph(TM), GMS v 3.32  
SUBIMAGE: Subtract section B from section A. 28-Oct-21 11:42:19

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Last update: **2023/11/01 20:18**

