

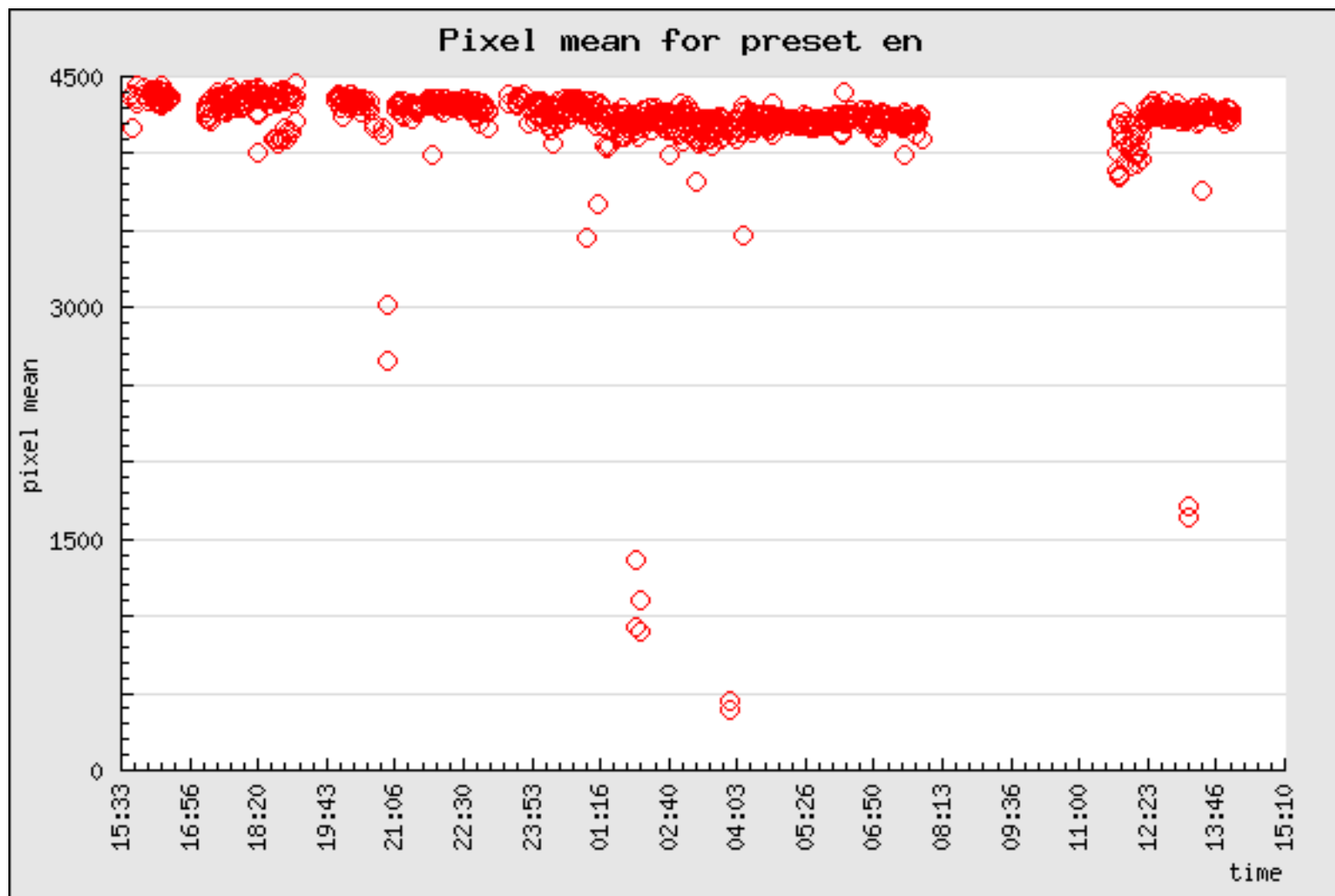
Krios Intensity Issues

Some data follows showing plots of beam intensity against time during data collection on various combinations of microscope/camera.

- Krios/FalconII combination had some experiments where intensity was constant (14mar13d) and some where it was varying semi-systematically (14mar28e). All attempts to reach high resolution were unsuccessful – is this linked to the beam intensity fluctuations?
- After FEG tip change on Krios two datasets (14mar28e, 14jun07c) were acquired using Krios/FalconII and both had well behaved beam intensity. At least one of these data sets has yielded a reconstruction below 4Å.
- Camera switched from FalconII to K2
- Data acquired on Krios/K2 now shows similarly bad behaved beam intensity (14jun20b)
- Health monitoring software installed. No issues observed.
- Beam intensity tests on the Krios/K2 (14jun25, 14jul01a) now show a relatively constant grid.
- ?? Return to data collection and wait and see?

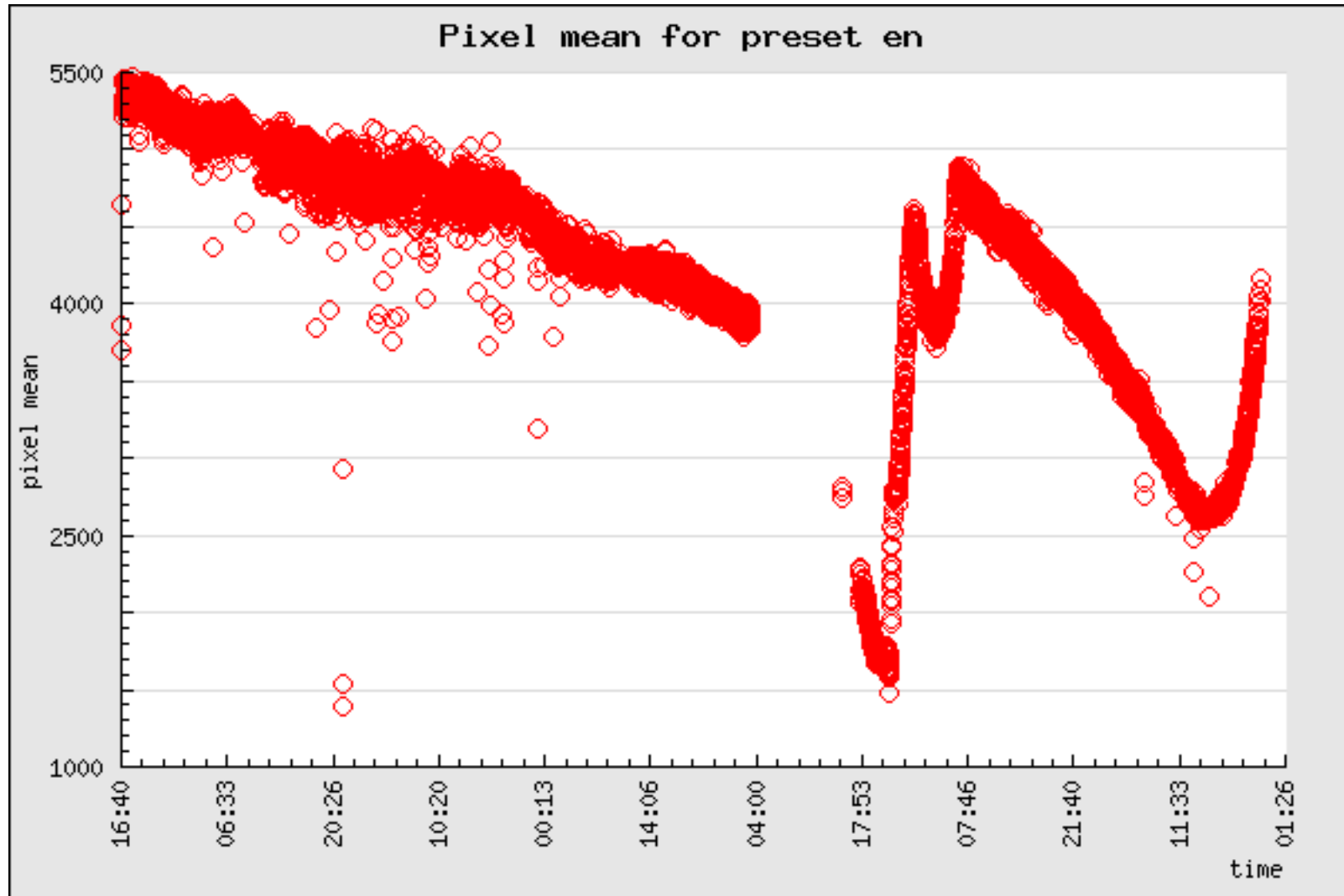
Seems like a similar issue was observed for the Polara in James Conway's lab. See last slide.

Krios + FalconII
Cryo grid



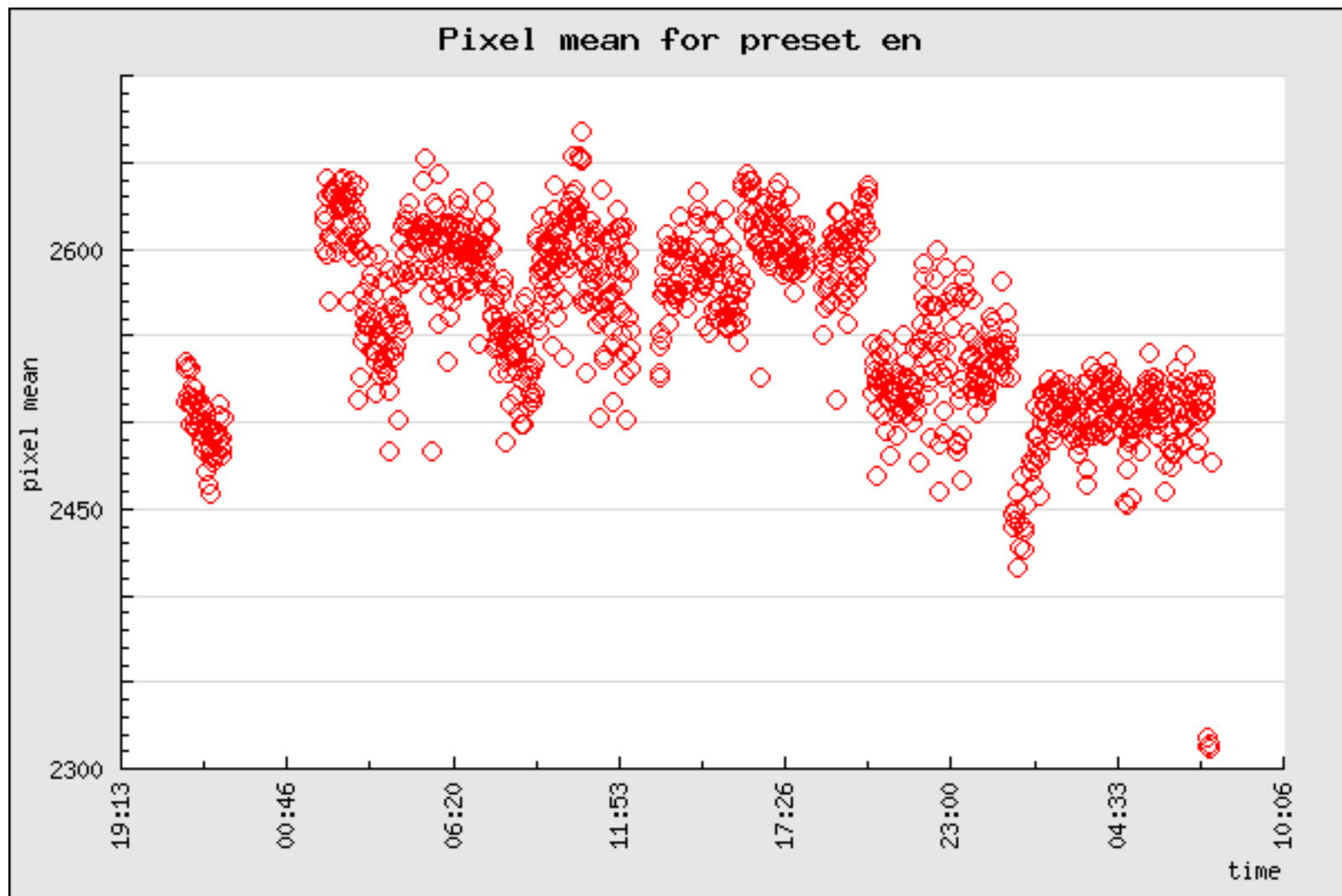
14mar13d

Krios + FalconII
Cryo grid



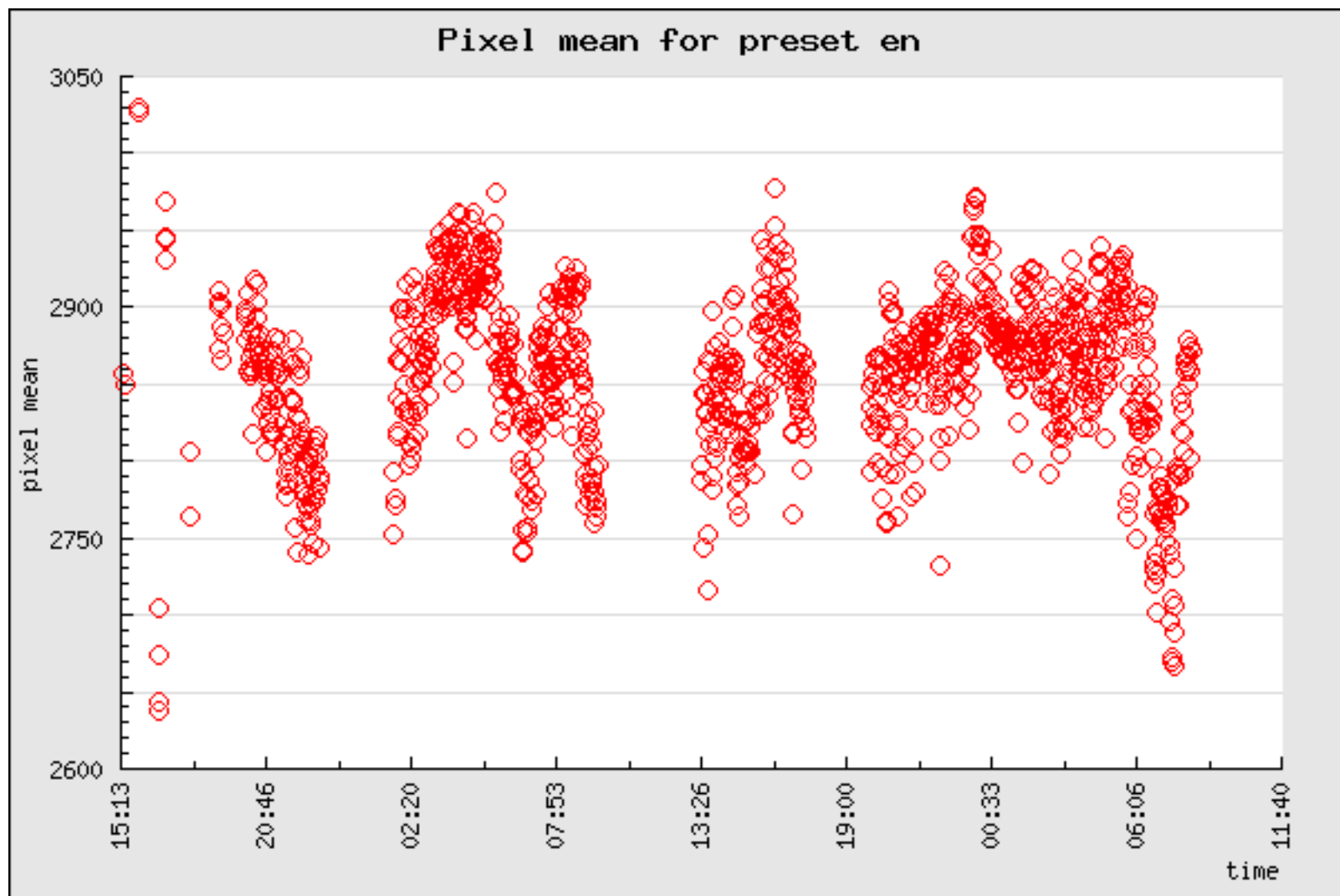
14mar28e

Krios + FalconII
Cryo grid



14mar25f

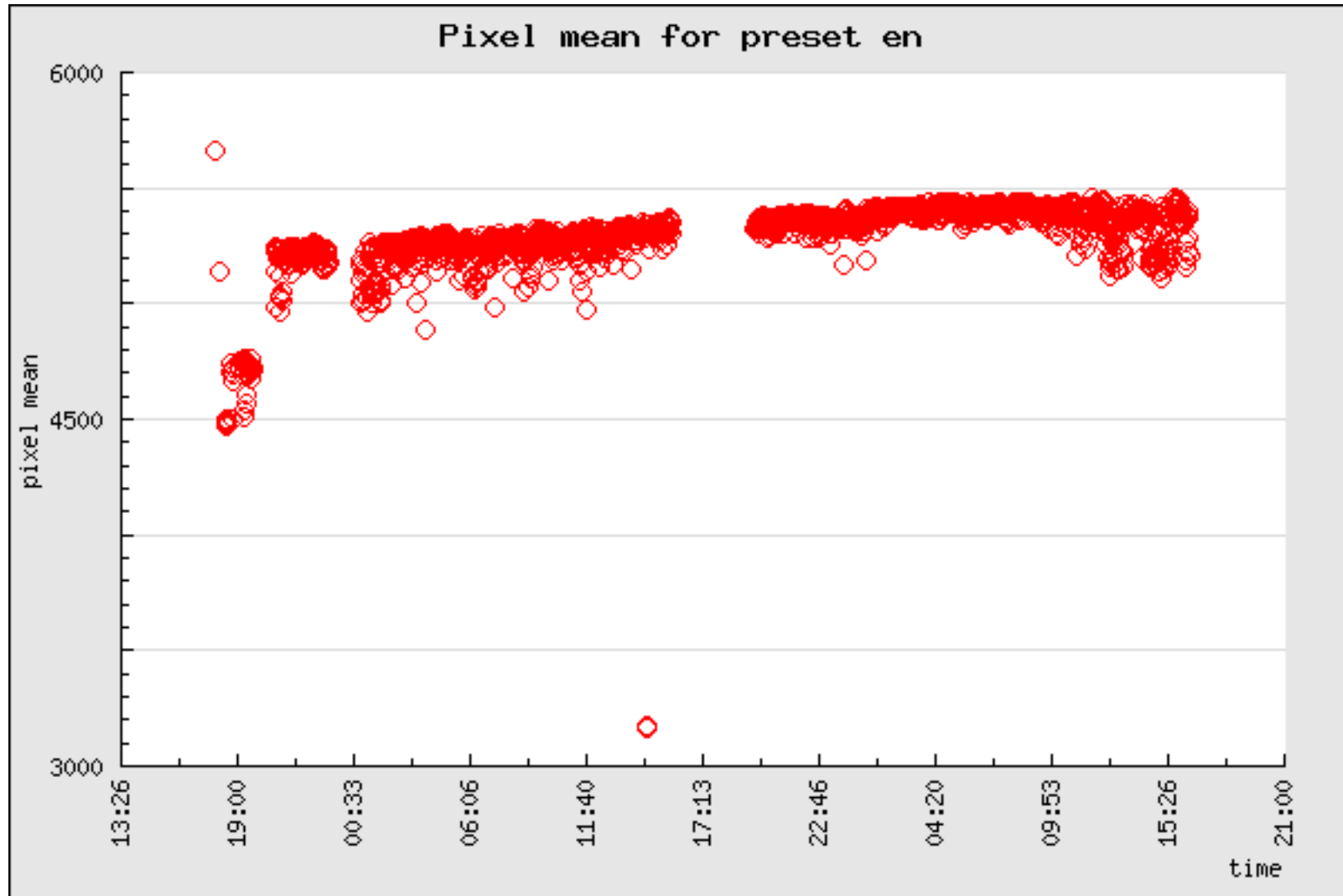
Krios + FalconII
Cryo grid



14mar21d

Krios + FalconII
Cryo grid

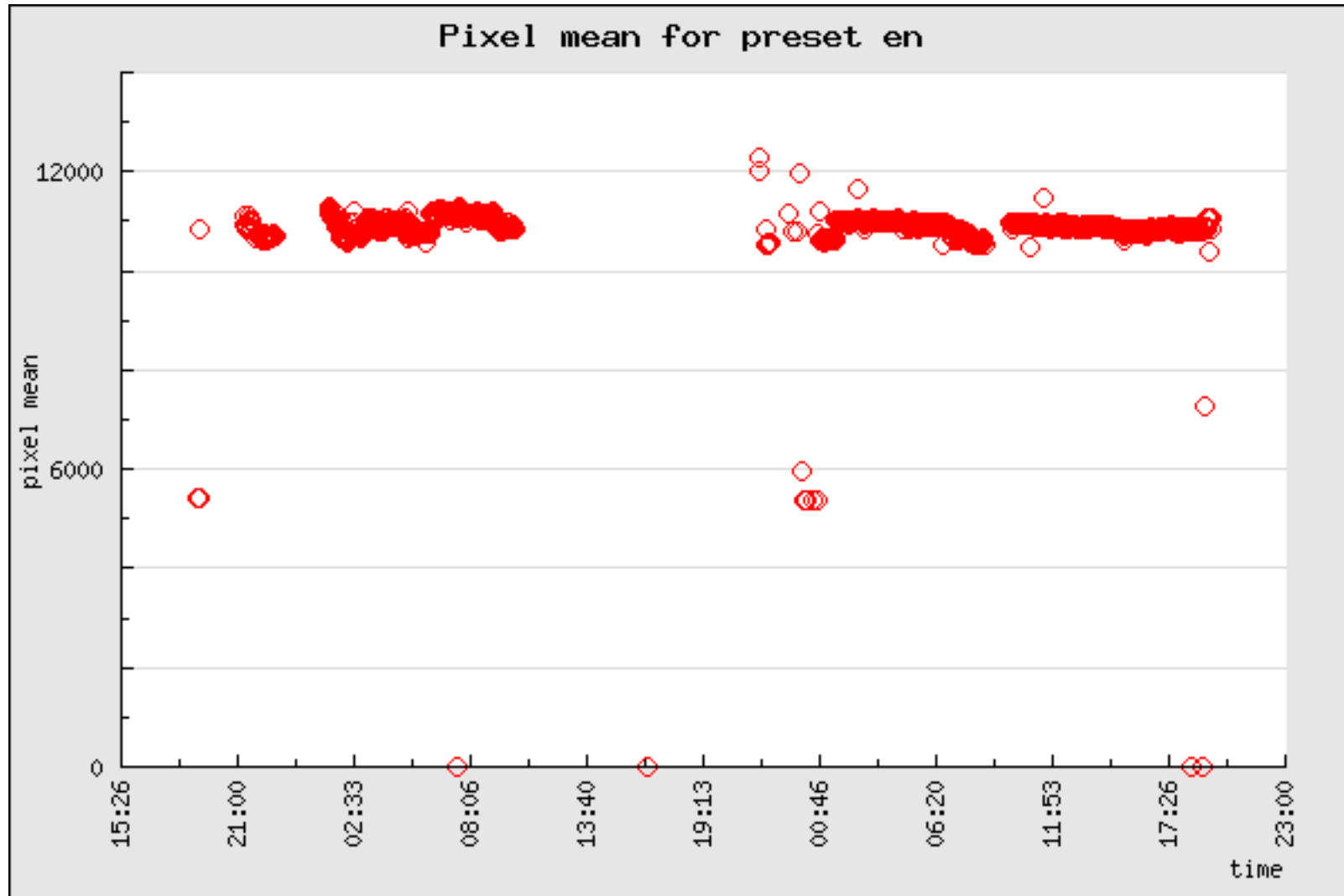
Immediately post FEG tip change



14jun05b

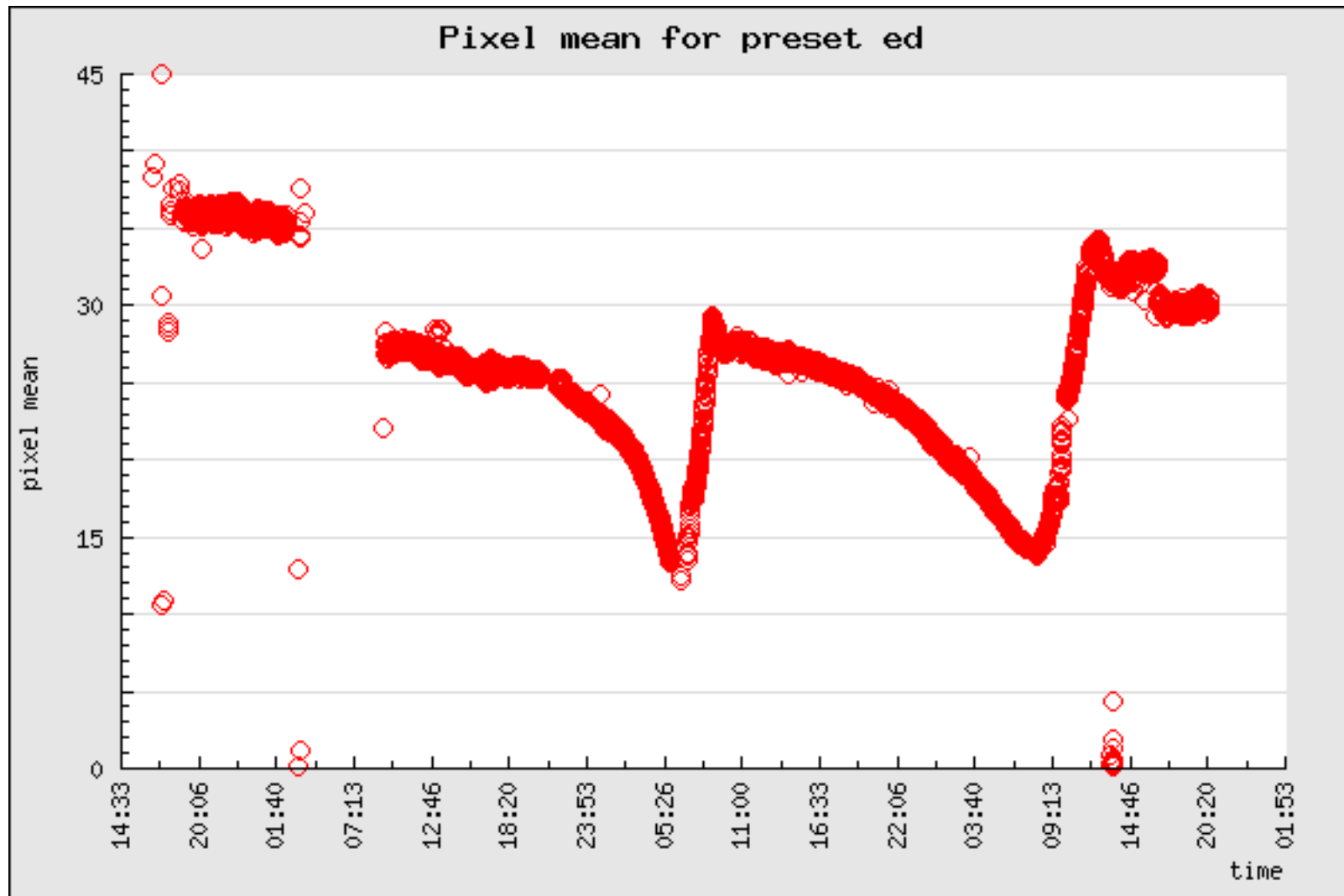
Krios + FalconII
Cryo grid

Immediately post FEG tip change



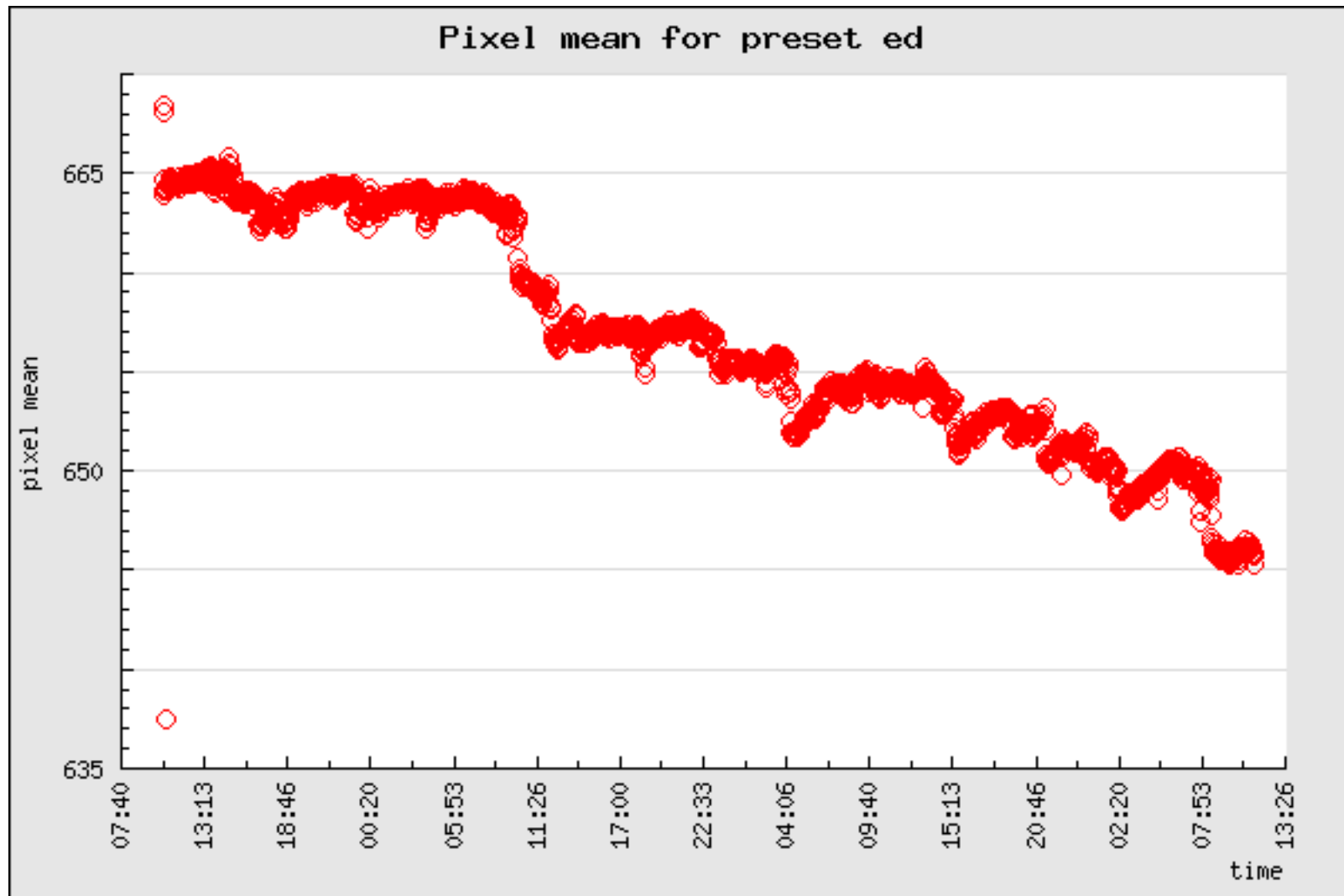
14jun07c

Krios + K2/counting.
Cryo grid



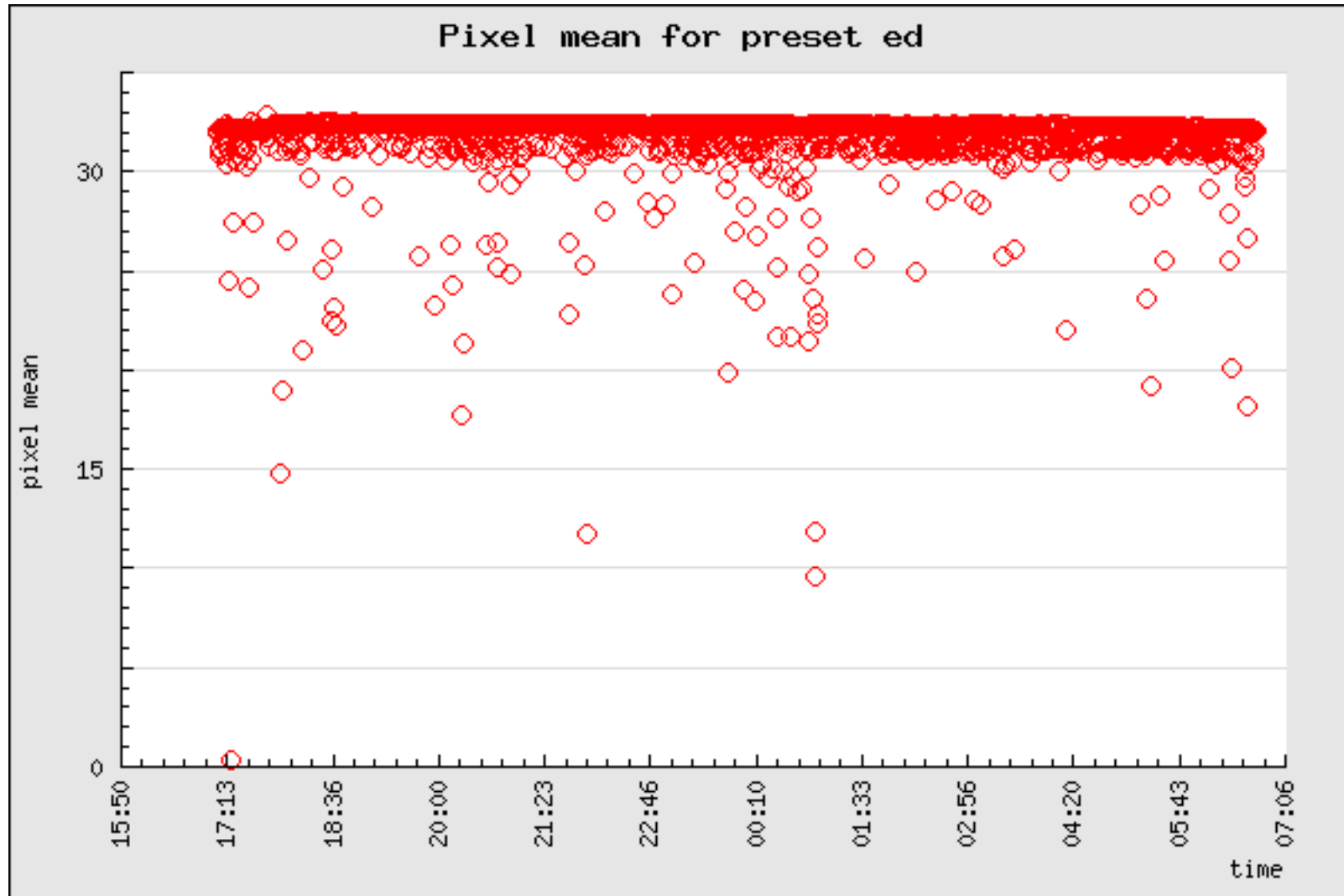
14jun20b

Krios + K2/counting.
Constant region of grid for testing



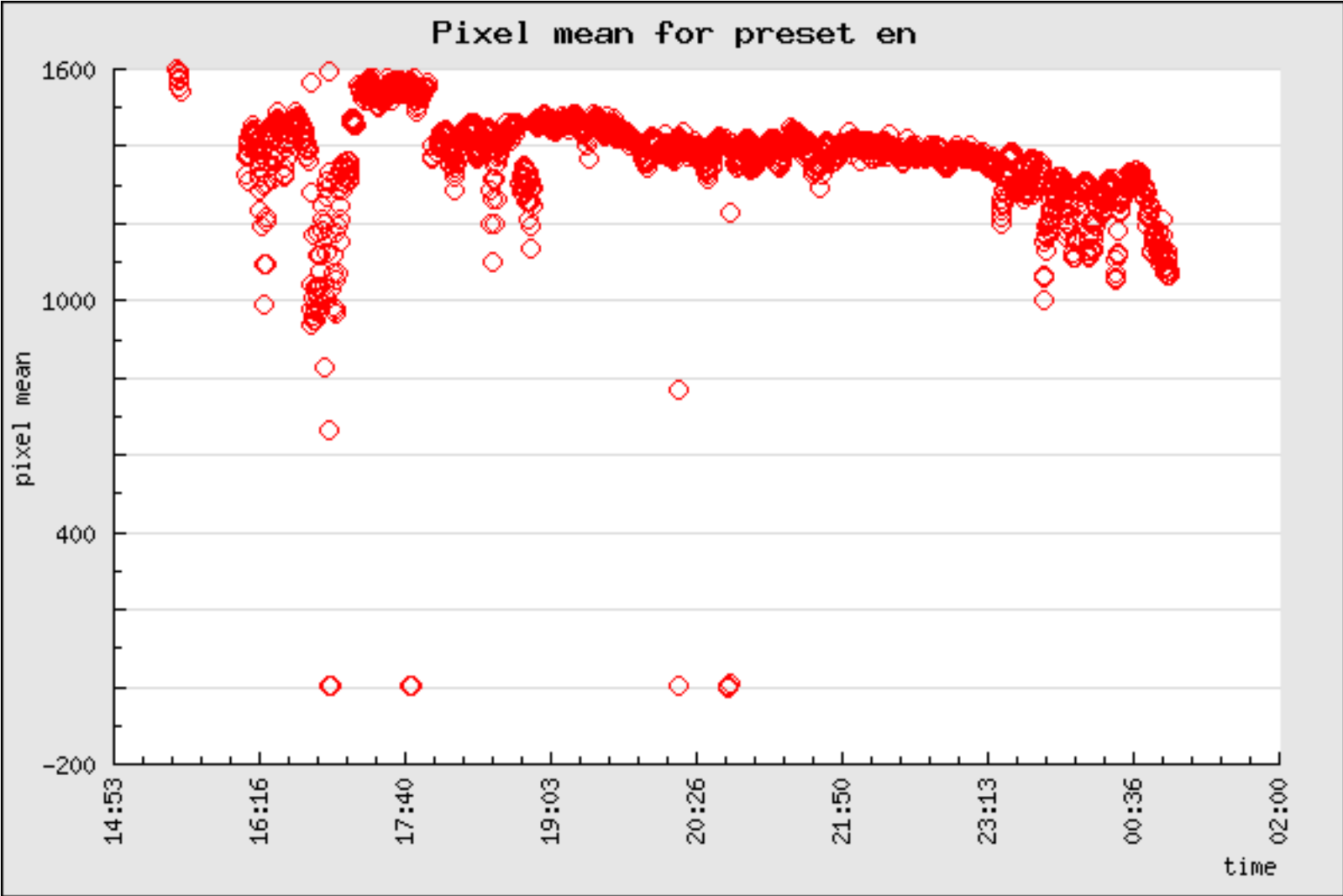
14jun25e

Krios + K2/counting.
Varying region of grid for testing, MSI mode



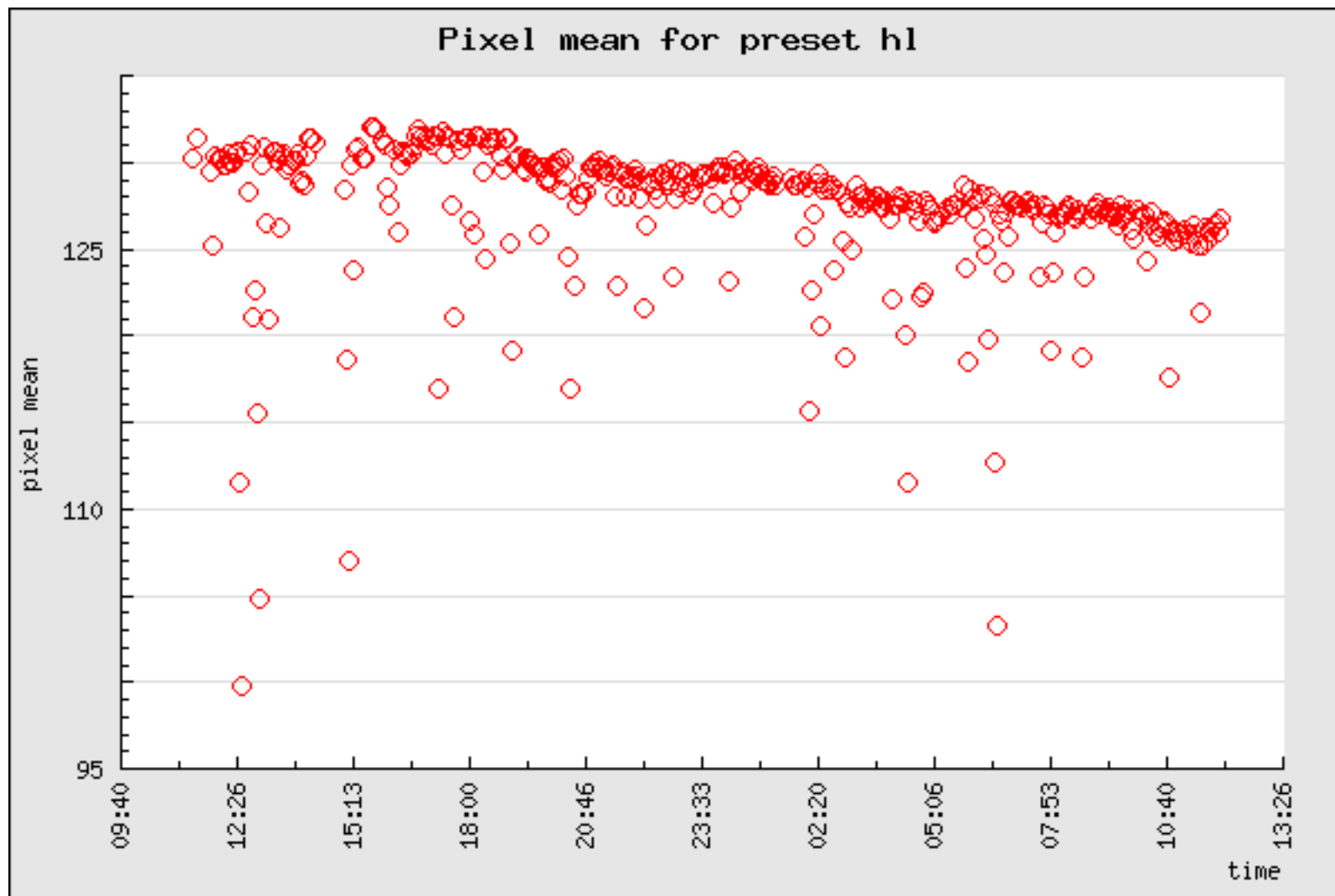
14jul01a

F20 + CCD.
Cryo grid



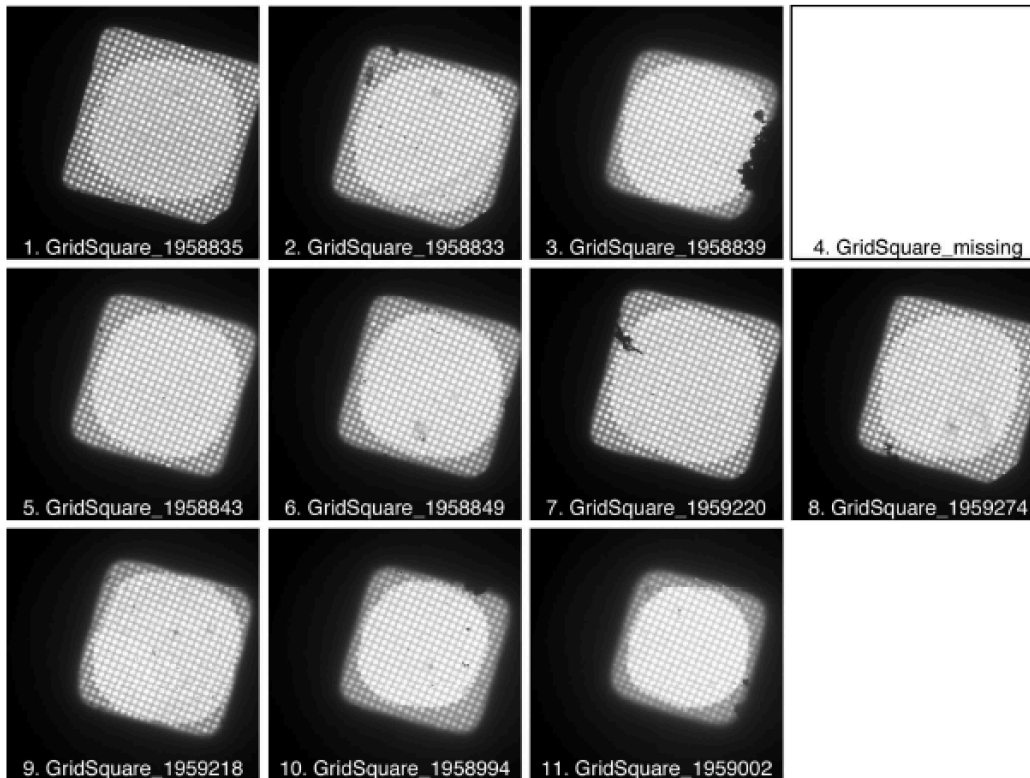
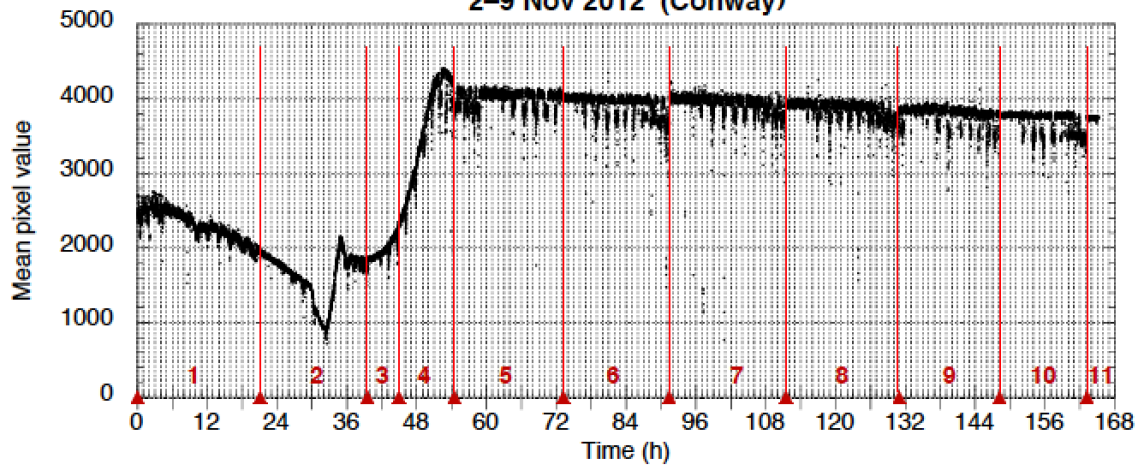
14apr23b

F20 + K2/counting.
Cryo grid



13sep27a

V-C sample #2 (FH) - image means (Vos)
2-9 Nov 2012 (Conway)



James Conway:

I had several visits to Acht for Polara and on one we noticed several times that the images were suddenly different in brightness.

EPU was running in manual mode: we had pre-picked grid-squares and holes, all the software had to do was visit each hole for imaging.

(In automatic mode it would choose holes based on brightness,

re-calibrating every 2 hours against an empty grid square - so it wasn't doing that). I then went back over all the images and plotted average brightness against time and

this showed the changes in brightness.

Obviously some images departed from those around it (edge of the grid, change of grid-square)

but there was so much data that a clear trend could be seen.

It wasn't catastrophic to my data analysis, but obviously a puzzle.

I assembled a series of plots and grid-square images. A subset is attached that shows the problem nicely, as well as other things:

1. Grid square times are marked and grid square images show nothing unusual with ice
2. The fluctuation is obvious at the start
3. The gradual decay in the last 2/3's is presumably ice build-up over 7 days
4. EPU was doing an odd spiral pattern for visiting holes, not terribly consistent, and the brief drops in average intensity were from areas of thicker ice at the edges of grid squares.

