doi:10.1017/S1431927622005037
Microsc. Microanal. 28 (Suppl 1), 2022

Not All Vacuum is Created Equal

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Cryo-electron microscopy (Cryo-EM) has transformed into a high-performance tool for routine determination of protein structures at near atomic resolution. This transformation has significantly increased the number of biomedical researchers requesting access to both mid- range (screening) and high-end transmission electron microscopes. To serve this increased demand, facilities aim to maximize available microscope data acquisition time, prevent downtime and minimize cryo cycle (warm up) time. To monitor and improve availability, we have developed a system vacuum monitor. The monitor consists of two parts. The first part collects benchmark data that allows operators and facility managers to check data collection speed and compare it to different collection strategies and software packages. The second part autonomously benchmarks hardware performance and advises on optimizing strategies.

Cryo-EM images contain a timestamp in their meta data. The first part of our monitoring system runs a dedicated script on the microscope computer to determine througput by counting the number of images taken in a certain timeframe and measure the overhead time the system needs between taking images (autofocus, stage moves, LN_2 filling, vacuum purges etc.) see Figure 1. The second part of our system autonomously monitors possible engineering issues and events that could significantly slow down data collection. Previously we described monitoring the room electromagnetic field cancellation and the LN2 autofilling systems [1].

In this study we will present case studies of the microscopes at the New York Structural Biology Center (NYSBC) where we have been continuously monitoring throughput and find opportunities to improve system performance. The first example is shown in Figure 2. A Pfeiffer Prisma Plus residual gas analyzer (RGA) and a Raspberry PI single board computer are connected to create a remotely accessible and autonomous system for monitoring leftover gasses in the vacuum [2, 3]. Once the vacuum in the microscope has a water content below a preset value the system can advise the facility operator to stop the cryo cycle or delay the next cycle for a certain period of time. The second example is shown in Figure 3. The graph to the left shows normal column Ion Getter Pump (IGP) pumping behavior, where the pressure in the microscope column is stable and below a set threshold. The right side shows oscillatory behavior where the IGP pump heats up due to contaminants in the vacuum causing the IGP current to increase too much. A flush of the IGP with room temperature N_2 gas or a heat up cycle of the IGP to temperatures between 50^o and 90^o C. typically gets rids of this erratic IGP behavior. The last example is presented in Figure 4. The top left shows a microscope where the vacuum buffer is purged as designed. A turbo molecular pump (TMP) expels gasses every eight hours. Bottom left shows a system with compromised vacuum (leak) where the buffer needs purging every half hour to maintain vacuum below triplevels. Note that cryo-EM images cannot be collected while the TMP is running as it negatively impacts resolution. As a results contant cycling of the TMP reduces microscope throughput.

All data is collected by a script that is started twice a day by the Windows operating system on the microscope platform to collect data from the microscope logging. The data is analysed by a python script on a server on the network. This setup allows installation of the monitoring system independent of facilities infrastructure and independent of different data collection software packages (Leginon, EPU, SerialEM, etc.) and the patterns can be analyzed by standard algorithms. An obvious next step is to use machine learning network to identify even deeper patterns that indentify present or imminent problems with the hardware.

We will present the design of these sytems and demonstrate how they help improve system availability and efficiency at the Simons Electron Microscopy Center, home to nine autoloader systems [4].

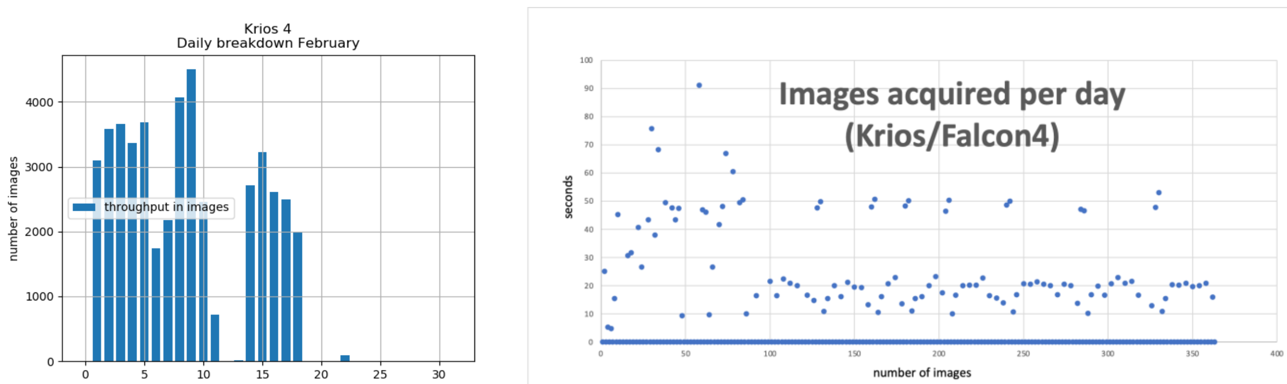

Figure 1. Analysis of time between acquired images for a Krios with a Flacon4. Overhead time is grouped in various timeslots as a result of different workflow steps (autofocus, beam normalization etc.)

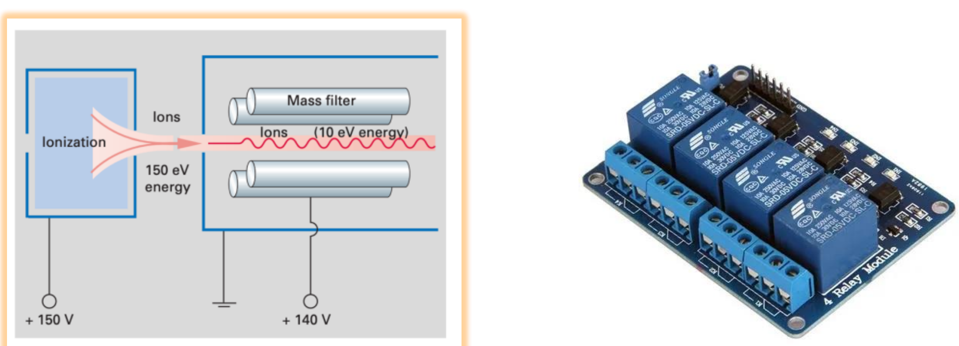

Figure 2. Left: Schematic of the Pfeiffer residual gas analyzer (RGA). Residual gases are ionized, and the mass filter counts the molecules for a given mass in a time interval. Right: A set of relays was added to privide remote and automnmous control of the RGA electronics. The RGA remains powered off during data collection to avoid interference.

https://doi.org/10.1017/S1431927622005037 Published online by Cambridge University Press

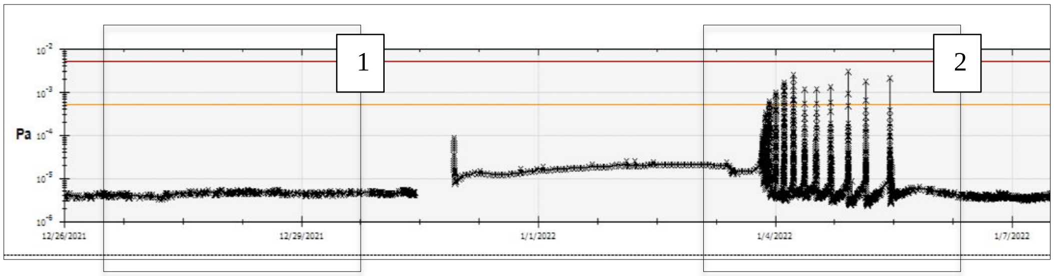

Figure 3. (1) Normal Colum IGP pump behavior (IGPco). (2) Oscillatory behavior where the pump continuously heats up due to malfunction and degrades the vacuum. The IGP is switched off by the IGP controller at a certain triplevel is reached at which point the cycle starts over.

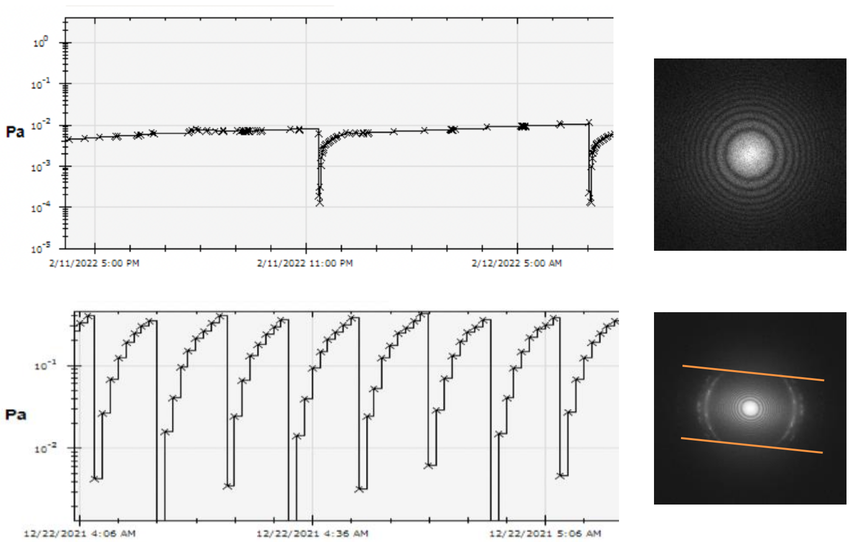

Figure 4. Top: Typical vacuum buffer cycle where the system turbo pump (TMP) expels gasses every eight hours; no loss of resoltiion is observed. Bottom: Compromised vacuum where the buffer cycle runs every half hour has a negative impact on data resolution as high frequency imfaormation is cut off. The high number of purges also impacts throughput.

References:

[1] Lambertus Michael Alink, Robert Gheorghita, Kashyap Maruthi, Anchi Cheng, Edward Eng, Clinton S. Potter and Bridget Carragher; 772 - System Evacuation Metrics Collector for IGP and cryo-cycle performance management (SEMCi) (2021)
[2] https://www.pfeiffer-vacuum.com/productPdfs/PTM06241111.en.pdf
[3] https://www.raspberrypi.org/
[4] Funding support from the Simons Foundation (SF349247), NYSTAR, and the NIH National Institute of General Medical Sciences (GM103310).