Streaming to RAM at High Speed with the Kinetix sCMOS in Micro-Manager

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Introduction
The Kinetix sCMOS is supported in the free, open-source software Micro-Manager. This enables the Kinetix to be used alongside the vast array of other microscope and imaging hardware supported in Micro-Manager. However, the enormous increase in speed that the Kinetix offers makes unique demands on Micro-Manager. To acquire at the very fastest speeds of this camera, there is some initial setup that is required, and some limitations to be aware of.

Until recently, high-speed acquisition was considered an acquisition running at about 1GB/s which corresponds to a 3200x3200 sensor resolution running at 50 fps in 16-bit mode. The Kinetix, on the other hand, provides data throughput of 1.7GB/s in 12-bit Sensitivity mode (88fps, 3200x3200px) and approx. 5GB/s in 8-bit Speed mode (498.5fps, 3200x3200px). We therefore are asking more from Micro-Manager than ever before.

What Can Micro-Manager Do?

Streaming to RAM
On a computer that meets our recommended specification, Micro-Manager is capable of streaming to RAM (memory) at the full speed of the Kinetix in all modes. Not all of the computer’s RAM is available to Micro-Manager for acquisitions. Typically around 50% of the available RAM is accessible for high speed acquisition.

For example, a computer with 64GB of RAM may be able to stream 32GB of data uninterruptedly at the full 500fps speed of the Kinetix. In 8 bit ‘Speed’ mode, at 10MB per frame, this would represent 3,200 frames, around 6.5 seconds of constant acquisition. In 12-bit ‘Sensitivity’ or 16-bit ‘Dynamic Range’ modes at 20MB per frame, 32GB would represent 1,600 frames (18 seconds in ‘Sensitivity’ or 19 seconds in ‘Dynamic Range’).

Streaming to Disk
Computer specification allowing, the 12-bit ‘Sensitivity’ and 16-bit ‘Dynamic Range’ modes are able to stream at full speed to disk, allowing constant acquisition limited only by disk capacity. But due to limitations in the internal architecture of Micro-Manager, this software currently isn’t able to match the full speed of the Kinetix in 8-bit ‘speed’ mode while streaming to disk. On testing PCs meeting the spec for full-speed streaming to disk, our engineers have found limitations between 1.5 and 2.0GB/s in writing to disk.
The data rate of the Kinetix must therefore be limited either through using regions of interest to reduce frame size, increasing exposure time or using external triggering to reduce the frame acquisition rate.

We are currently working on a solution to enable full-speed streaming to disk in Micro-Manager. In the meantime, for 8-bit ‘Speed’ mode streaming to disk, please contact us for an alternative free solution developed by Teledyne Photometrics.

How To Set Up Micro-Manager For ‘Speed’ Streaming to RAM

Step 1: Installing the correct Micro-Manager Version

Our fix to Micro-Manager to enable high-speed streaming with the Kinetix is included in the latest nightly builds of Micro-Manager 2.0gamma.

Please use a version of 2.0gamma from the 4th of March 2021 onwards.

To see the list of versions, please follow this link.

Note: if you are constrained to use particular versions of micro-manager due to limited compatibility with other hardware, please contact us.

Installing the Kinetix into Micro-Manager

For instructions on installing the Kinetix into Micro-Manager, please consult our Micro-Manager guide available on our website.

Step 2: Setting up the Sequence buffer

Once you have Micro-Manager installed and your required hardware configured, there are a few settings to enable high speed acquisition to RAM. The first is the Sequence Buffer size.

The sequence buffer holds frames that come from the camera (via the device adaptor), before they are then copied to and processed by the Java environment (ImageJ). At its full field of view, the Kinetix is delivering frames to the buffer up to every 2ms (at 500fps), but the copying to Java takes longer than this, so the sequence buffer will fill while frames wait to be copied.

The capacity of the Sequence buffer can be set via Micro-Manager → Tools → Options → Sequence Buffer Size:

The default size is 250MB. With slow acquisitions in MDA mode, or with single snaps, the buffer is not used at all and the images are copied to the Java memory directly.
Configuring Sequence Buffer size
To maximize the possible acquisition data size, the sequence buffer should be set to around 40-45% of available computer RAM.
For a given maximum acquisition size calculated below, the Sequence Buffer size should be set just slightly smaller (90%) or the same size.

The acquisition size is given by: Image pixel count × Bytes per pixel × Number of frames.

For example, on a PC such as HP Z4 G4, streaming 1,200 8-bit images at 3200x3200 to RAM (12GB of data) requires setting the Sequence buffer to at least 10GB size. With a smaller size such as 8GB, the buffer may overflow and acquisition may fail due to slow copying to the Java memory.

In the case of sequence buffer overflow, the following error message will appear:

![Error Message]

Step 3: Setting the ImageJ Memory
The ImageJ Memory is where images are copied to for processing, displaying or saving to disk from the Sequence buffer.

The memory size can be set via ImageJ → Edit → Options → Memory & Threads.

Configuring ImageJ Memory Size
To maximize the possible data acquisition size, the ImageJ Maximum Memory should be set to around 45-50% of the computer’s memory.

The computer’s total RAM, minus the Sequence Buffer, and minus the ImageJ Memory, must leave enough RAM for the operating system to run, and any other programs that must be running for your imaging setup. This will vary from computer to computer depending on available hardware, Windows versions, applications required etc., but is likely to be around 4GB.

Step 4: Running an Acquisition
Set up your experiment in Micro-Manager as normal. Check that the ‘total memory’ for the acquisition provided in the Mutli-Dimensional Acquisition Window is below the maxima you set in Step 3, and the same or only slightly larger than the Sequence Buffer size in Step 2.
Keeping Up: How To Monitor Your Acquisition

During an acquisition
If you are acquiring at high speed, the Sequence Buffer is expected to fill up very quickly. This can be monitored via Micro-Manager → Plugins → Developer Tools → Sequence Buffer Monitor, as shown below, left. This should not reach 100% during acquisition.

The utilization of the Java memory can also be monitored via ImageJ → Plugins → Utilities → Monitor Memory, below right. Similarly this should stay below 100%.

Checking that the acquisition completed successfully
Once an acquisition has completed, select the image window and open the ‘Image Plane Metadata’ section of the Image Inspector, as shown below.

Your total frames should be the same as the PVCAM-FMD-FrameNr and PVCAM-FrameNr. This should also be equal to the ImageNumber + 1, as the first frame has an ImageNumber of 0.

If the metadata frame numbers are larger than your requested number of frames, frames were acquired by the camera that the computer was not able to record, and those frames are lost. To avoid frame losses, you may need to reduce the total acquisition size or reduce the acquisition speed or data rate.