LOW FORM FACTOR, HIGH STABILITY

In life-sciences research, cooling biological samples of, for example, proteins, viruses and bacteria to extremely low temperatures facilitates new forms of microscopy. This requires the integration of a sample holder provided with a cold stage into the workflow of preparing and analysing samples. Demcon kryoz designed an efficient cryogenic micro-cooler that has a low form factor and demonstrated its high mechanical and thermal stability.

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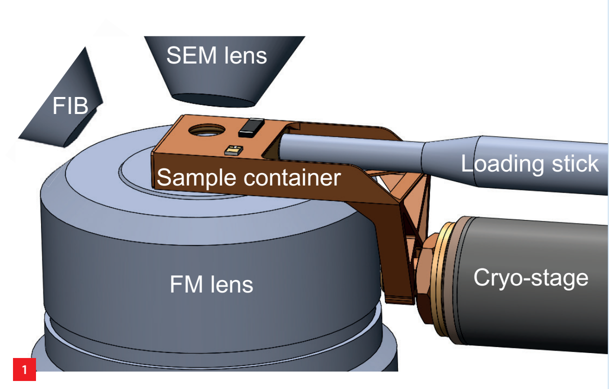
One of the upcoming techniques in life-sciences research is CLEM (correlative light and electron microscopy), which combines light microscopy and electron microscopy. The two modalities are complementary to one another due to their different length scales: nanoscale electron microscopy provides high-resolution images of a sample while microscale light microscopy can be used for identifying regions of interest in the sample.

In a CLEM system, the sample is imaged using an electron beam and an optical light path simultaneously. This ensures that no changes have occurred in the sample during the analysis, as could be the case when the two microscopy modalities are used consecutively. Overlay of the two images is thus achieved automatically. Cooling to cryogenic temperatures is often used to fix (vitrify) the sample in order to obtain the highest resolution in imaging.

Delmic, located in Delft (NL), developed a fluorescence microscope (FM) that can be integrated with a scanning electron microscope (SEM). If the SEM is additionally provided with a focused ion beam (FIB) for preparing samples before imaging, the resulting system comprises three 'lenses' centred around the sample (Figure 1). This restricts the available space for a sample holder fitted with cooling infrastructure. Nevertheless, it has to have high mechanical and thermal stability to achieve high-resolution imaging. As part of a joint development project, Demcon kryoz and Delmic collaborated to design and realise a cryo-FM/FIB/SEM using Demcon kryoz' novel cryogenic micro-cooler technology.



Schematic of a cryogenic sample holder for a microscopy system integrating three modalities: scanning electron microscopy (SEM), fluorescence microscopy (FM) and focused ion beam (FIB).

Design

Challenging requirements were defined for the cryogenic micro-cooler; see Table 1. Given the limited form factor and the long standing time, an efficient cooling solution was needed. For this, the Hampson-Linde cycle was selected, which is commonly used for the liquefaction of gases, in a regenerative cooling process that relies on the Joule-Thomson effect. Upon free expansion of a gas through a flow restriction, the gas cools and its temperature decreases. The advantage of this solution is that no compressor is required in the cooling device, i.e. there are no moving parts that can induce vibrations.

The actual implementation consists of a cooler chip that is made using lithographic techniques (Figure 2). Nitrogen gas is expanded from 95 to 1 bar, yielding a net cooling power of 200 mW at 80 K. No boiling liquid nitrogen is present in

Table 1
Main requirements for the cryogenic micro-cooler.

Sample temperature	≤ 108 K
Thermal stability	±20 mK (stationary)
Mechanical drift	≤ 4 nm/min
Vibration level (peak-to-peak)	≤ 1 nm @ 200 Hz
Degrees of freedom (DoFs)	5
Cool-down time	≤ 2 h
Standing time	> 6 h

AUTHOR'S NOTE

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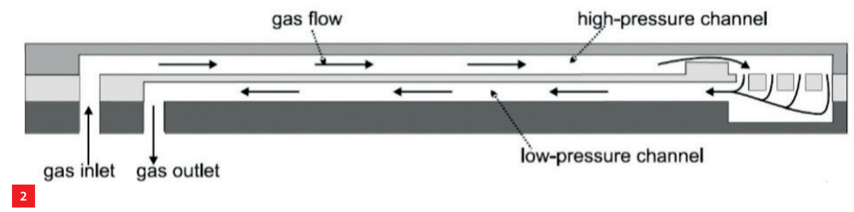
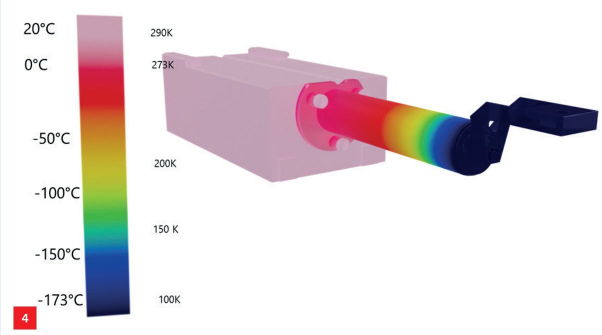


The cold stage with the flow restriction on the right.



Result of thermal LEM analysis of the cryogenic micro-cooler design.

the system during operation, which keeps vibrations low. A sample holder is mounted on the cold stage, which in turn is integrated with cooling infrastructure (nitrogen high-pressure gas supply) into a complete micro-cooler. For manipulation of the sample, i.e. to position it with respect to either of the three 'lenses', the micro-cooler is mounted on a 5-DoF motion stage. Figure 3 shows the design of the micro-cooler and its mounting on an example stage.

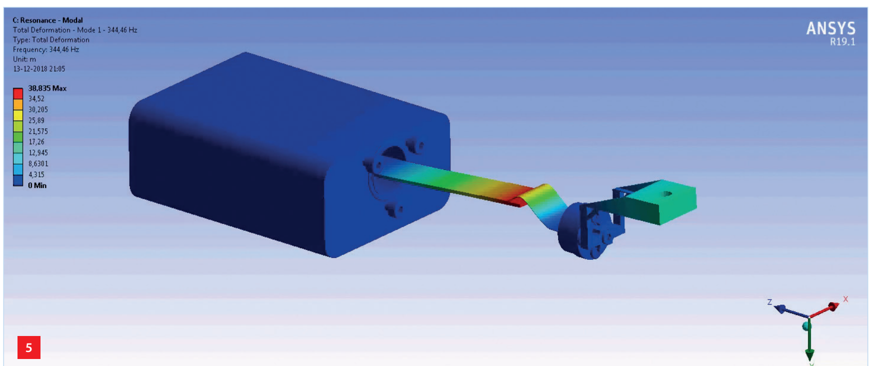
Thermal analysis of the complete system was performed using lumped-element modelling (LEM). Demcon kryoz has developed a dedicated LEM toolbox for use in the popular simulation environment Simulink, for modelling thermal, fluidic and vacuum systems to predict their (real-time) dynamic behaviour. It comes with a LEM library containing a large number of predefined modelling blocks that combine physical models with actual (off-the-shelf) part performance data, making detailed dynamic modelling of complex systems possible.

A schematic result of the LEM analysis is shown in Figure 5. It confirms that only the sample holder itself is cooled down to, for example, 100 K, while the cooler's base remains at room temperature. The large temperature gradient extends over a small distance (about 50 mm), which makes it possible to provide all mechanical interfaces to the cooler at room temperature, while the cryogenic temperature is only produced at the location where it is relevant, i.e. on the sample.

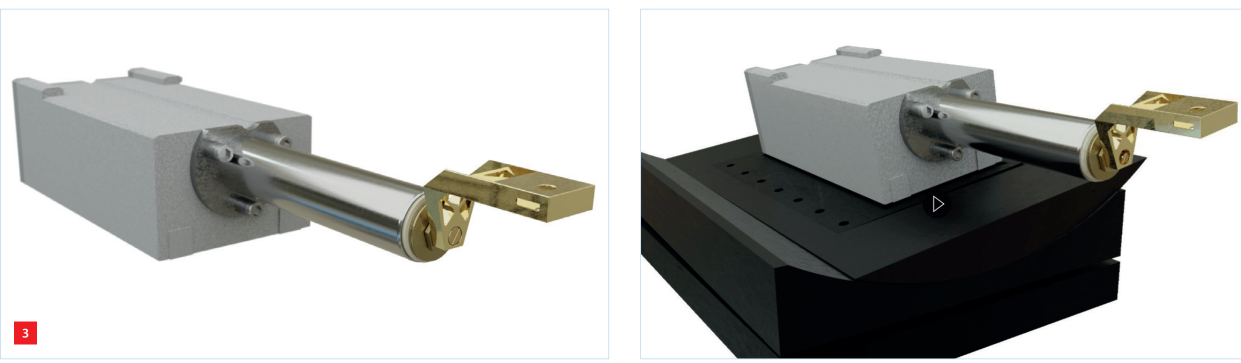
Verification

Testing of the mechanical and thermal performance was conducted at the Max Planck Institute of Molecular Physiology in Dortmund, Germany (Figure 6). The cryogenic micro-cooler was integrated in a dual-beam system, comprising a FIB for sample preparation and a SEM for analysis, while retro-fitted with an FM.

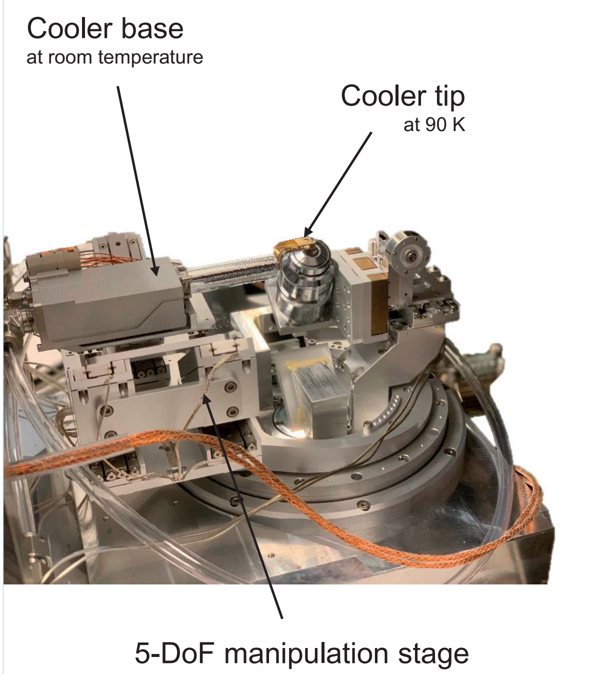
Peak-to-peak vibration levels for the cold tip (Figure 7) were found to be well below 1 nm over a wide frequency range up to 2 kHz, except for a peak at 50 Hz (probably a mains-related artefact). Hence, the requirement of ≤ 1 nm @ 200 Hz is amply met. Mechanical drift of the cold tip was measured to be below 3 nm/min, which satisfies the requirement of ≤ 4 nm/min. A duration test was performed to test the thermal stability (Figure 8), demonstrating the required ±20 mK, and the standing-time, for which 6 h was specified.



Modal analysis of the micro-cooler, showing the first mode and demonstrating the stability of the cold tip, of which the amplitude remains below 1 nm.



Design of the cryogenic micro-cooler. On the right, a still from an animation of the cooler mounted on a moving 5-DoF manipulation stage.



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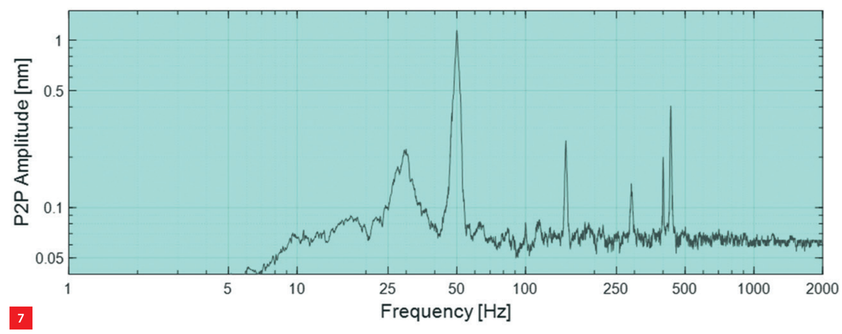
Set-up for verification testing of the cryogenic micro-cooler.



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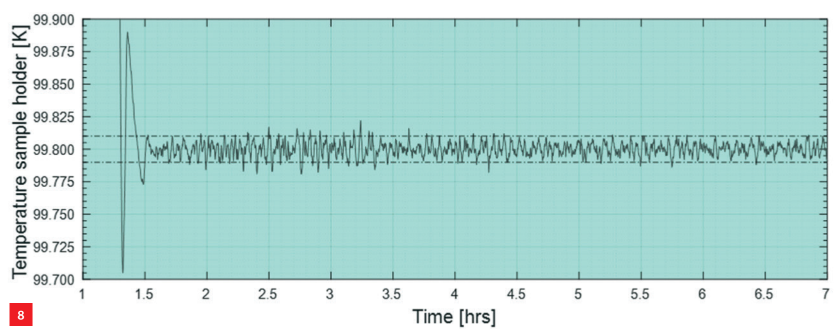
Mechanical performance of the cryogenic micro-cooler cold tip: peak-to-peak vibration amplitude as a function of frequency.



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Thermal performance of the cryogenic micro-cooler cold tip: sample holder temperature over time. The dashed horizontal lines indicate a ±20 mK range.

Conclusion

A cryogenic micro cold stage has been designed that satisfies strict requirements concerning mechanical and thermal stability. It is a first milestone on the CryoEM roadmap being pursued by Demcon kryoz. New challenges include expanding the standing time to several working days and shifting the cooling regime down to 40 K and below. In addition, the micro-cooler has to be made suitable for transmission electron microscopy, next to SEM. This means that vibration reduction has to move from the nanometer down to the picometer level. And finally, applications for different types of microscopes each require their own form factor for the micro-cooler and its integration with different types of sample holders.