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To whom it may concern

expert evidence is provided for the **Cryo-electron microscopy and tomography core facility CEITEC Masaryk University** respective to the tender for the purchase of the **FIB-SEM microscope** based on the written request from Jiri Novacek from August 3rd, 2020.

Technical specifications

The subject of the tender is a purchase of a unique Helios Hydra CX DualBeam System for room-temperature and cryo-electron microscopy applications as well as correlative light and electron microscopy applications for life-science research. The instrument has a high-resolution SEM column and an inductively coupled plasma focused ion beam source with up to four different ion species and fast exchange of the operating ion source. In addition, the microscope will be equipped with a cryo-stage and a cryo-lift-out manipulator for removal of lamellae from bulky high-pressure frozen life-science specimen. The system has a gas injection system (GIS) for metal sputtering inside the microscope chamber, and most importantly, a cryo-fluorescence light microscopy module for correlative light and electron microscopy imaging inside the FIB-SEM microscope chamber under vacuum and cryo-conditions.

I am fully supporting the tender for the purchase of the Helios Hydra at CEITEC MU based on the detailed background research, market research, and personnel experience. I am in charge of one of the leading cryo-electron microscopy laboratories at the Max-Planck-Institute for Biochemistry in Martinsried (Germany). My laboratory is using at the moment three FIB-SEM DualBeam microscopes operating with Ga-Ions very similar to the subject of the current tender at CEITEC MU. Brief substantiation with respect to the unique character of the technology requested within the current tender is provided below, more detailed substantiation can be delivered upon additional request:

Substantiation for unique character of the technology

Cryo-electron microscopy is nowadays the only technology which can provide structural data on the macromolecular complexes at near-atomic level detail *in situ*. Sample preparation workflow requires processing (thinning or milling) of vitrified cells by FIB-SEM microscope followed by acquisition of cryo-electron tomography data with a transmission electron microscope. In order to target the areas on interest in the cell, additional fluorescence imaging in a separate cryo-fluorescence microscope is necessary. Until recently, the correlative process required transfer of the sample between the cryo-





fluorescence microscope and FIB-SEM microscope followed by the alignment of the data. In addition, standard FIB-SEM microscopes utilize only Ga-ions for the removal of the cellular material which significantly limits the time for thinning/milling and thus the size of the samples attainable for this workflow. Removal of material with the Ga-ions is a factor of 10 times slower than Plasma-ions (e.g. Ar, Xe, O and N.) The Helios Hydra instrument from Thermo Fisher Scientific is a unique instrument equipped with four species of inductively coupled plasma focused ion beam source and an integrated cryo-fluorescence module inside the microscope chamber. This unique combination will allow the researchers at CEITEC MU to efficiently prepare the cellular lamellas at the areas of interest and focus their research on larger cellular systems and especially on more complex specimen like organisms and tissue. Thermo Fisher Scientific is currently the only supplier able to offer a plasma-based cryo-FIB-SEM microscope with an integrated cryo-fluorescence module and field-proven technology for the entire integrated lamella milling and tomography workflow.

Substantiations, that other similar technologies cannot be used

To my knowledge and based on my background research, there is no company other than Thermo Fisher Scientific providing a cryo-fluorescence module which can be incorporated in the chamber of a FIB-SEM microscope. The company Delmic has an in-chamber fluorescence module which, however, is not yet designed for operation at cryo-conditions, and thus such a solution cannot be used for cryo-electron microscopy.

To my knowledge, no company developing FIB-SEM microscopes other than Thermo Fisher Scientific currently offers multi-species inductively coupled plasma focused ion beam source. Tescan company produces inductively coupled plasma focused ion beam source with only single ion species and does not offer a product with integrated cryo-fluorescence microscope.

Therefore, the solution provided by Thermo Fisher Scientific company is the only technology available on the market which can fulfil the requirements for the flexibility in plasma sources for fast and appropriate sample thinning/milling and furthermore for the in chamber correlative light and electron microscopy imaging at cryo-conditions.

Pricing of acquired technology

I have read the quotation provided to CEITEC MU for the purchase of the Helios Hydra CX DualBeam System and I do confirm that the price is very competitive with the quotation I have personally obtained from Thermo Fisher Scientific for the instrument with similar configuration which I plan to purchase to my lab in the near future.

Yours sincerely,

Juergen Plitzko 

Martinsried, September 24th, 2020



