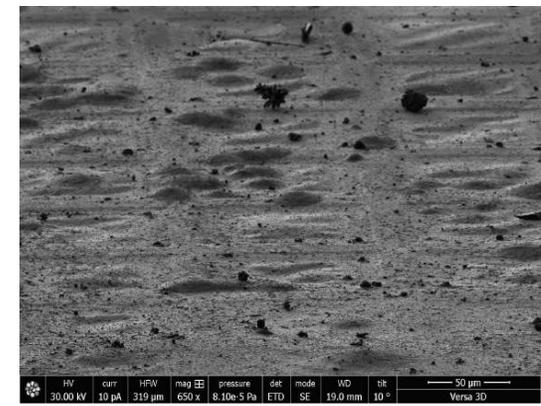
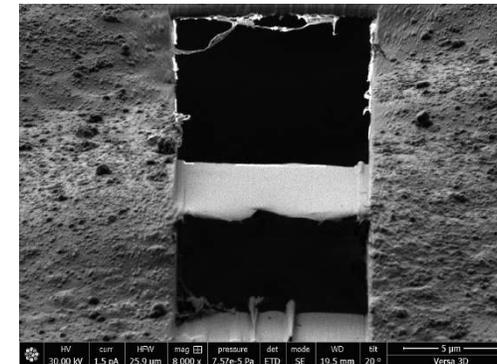
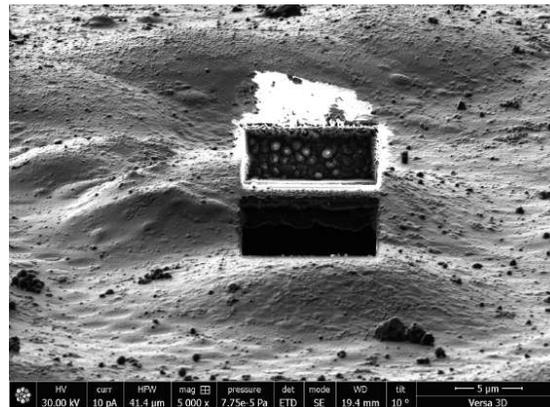


# Improved in situ visualization of regions of interest in eukaryotic cells using HPF and cryo FIB

Jirka Novacek (CEITEC-MU),  
Ludek Lovicar (IST Austria),  
Thomas Heuser (VBCF)  
24. 09. 2018



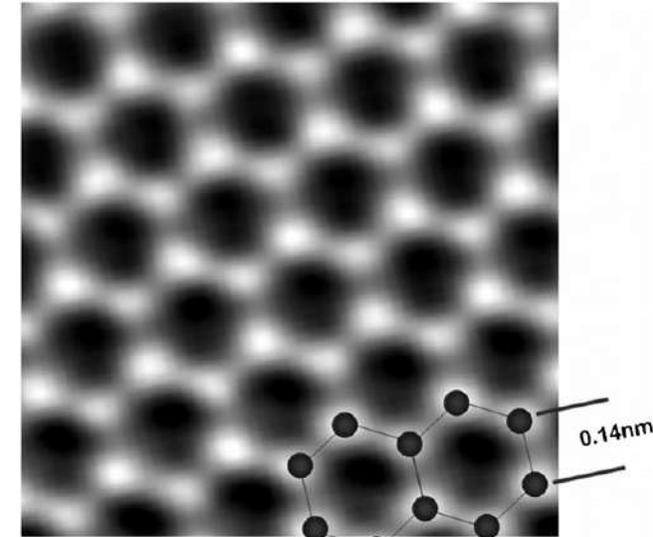
# Pilot Project Introduction



Electron Microscopy (EM) enables insights into atomic details ( $< 1 \text{ \AA} = 0.1 \text{ nm} = 1/10.000.000. \text{ mm}$ )

but biological samples need protection from dehydration (vacuum inside EM instruments) and electron radiation so that special sample preparation is needed which limits resolution

For large biological samples (e.g. cells) highest quality can be achieved by high pressure freezing (HPF), then “cutting out” the region of interest by focused ion beam (FIB) milling and cryo-TEM visualization



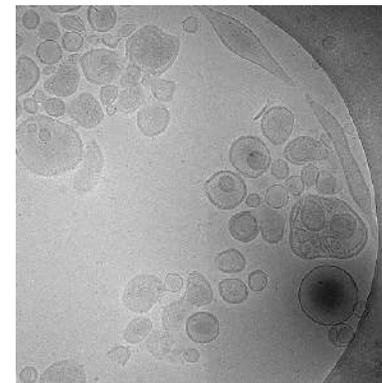
Dato et al., 2009

FEI Titan Krios, CEITEC

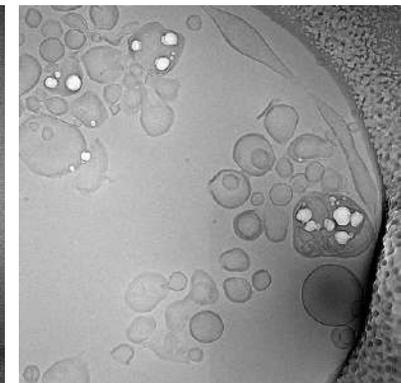
HPF Cryo-FIB



before radiation



after radiation



# Pilot Project Introduction



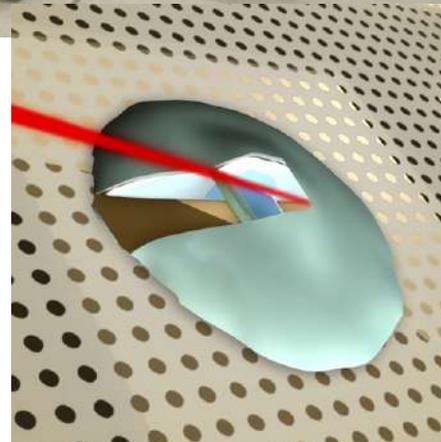
sample preparation by HPF:  
High Pressure Freezing

Cryo-FIB: cutting out the  
region of interest

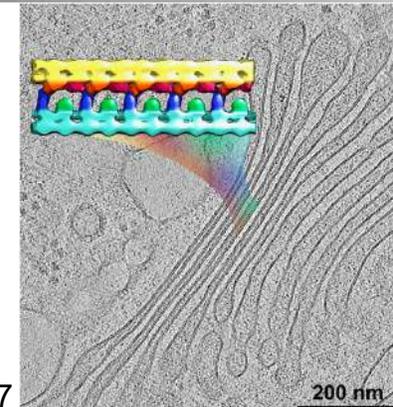
2D & 3D Visualization by  
TEM: Transmission  
Electron Microscopy



HPF Cryo-FIB



FEI



Burbaum et al., 2017

200 nm

# Project - Implementation



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Marlene Brandstetter  
Nicole Fellner  
Sonja Jacob  
Harald Kotisch



**IST-Austria:**  
**Ludek Lovicar**  
Vanessa Zheden  
Daniel Gütl  
Walter Kaufmann



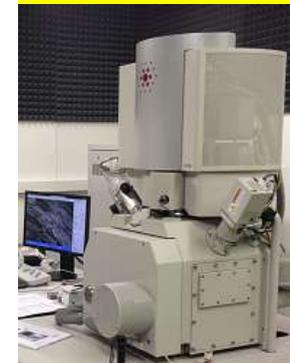
**CEITEC-MU:**  
**Jiří Nováček**  
Michal Babiak  
Matyáš Pinkas  
Anatolij Filimoněnko  
Radka Dopitová  
Jiří Nečas  
Pavλίna Kurková



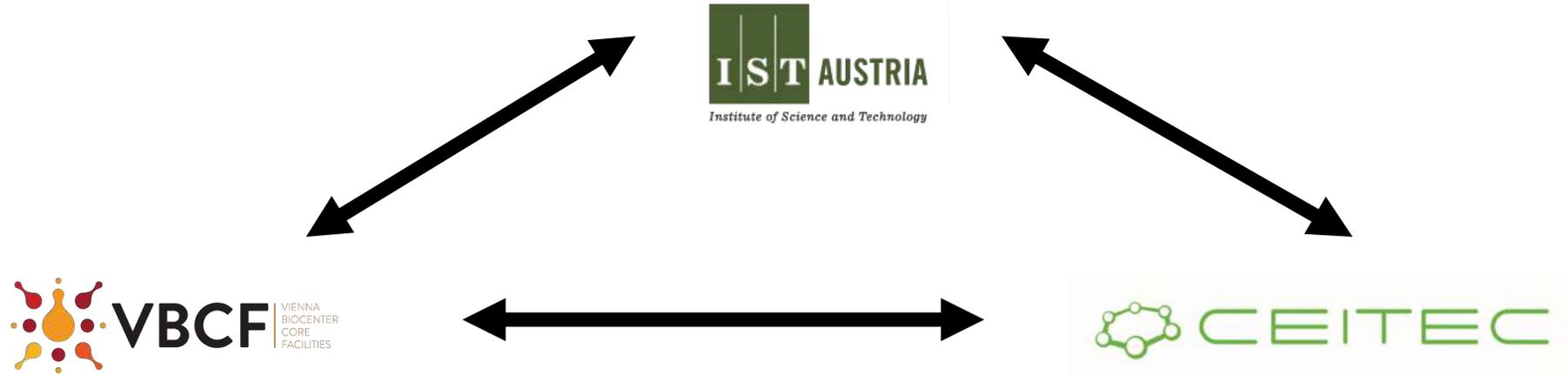
## ***HPF (High Pressure Freezing)***



## ***Cryo-FIB (Focused Ion Beam) Milling and TEM visualization***



# Project - Implementation



## Staff Exchanges / Knowledge Transfer:

partly done, partly ongoing

## Internal Workshops:

started, but delayed due to installation of new, critical equipment

# Project Results: HPF



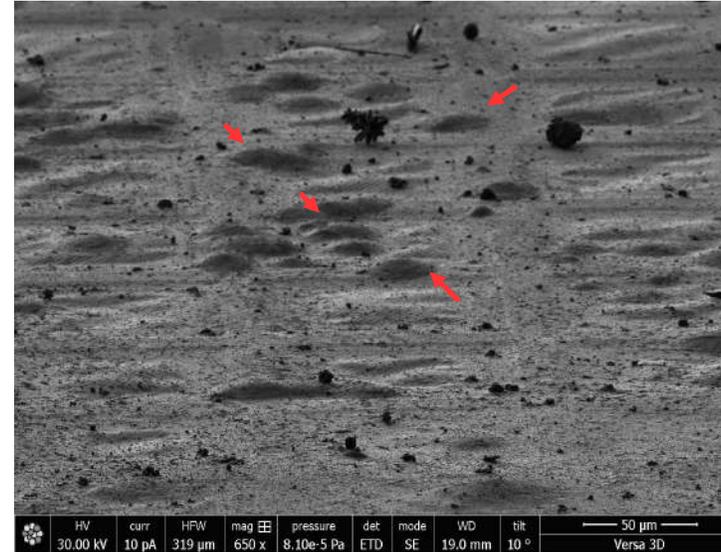
- **Comparison of different HPF instruments:**
  - > Leica EMpact preferred for cells containing viruses; otherwise Wohlwend Compact well suited
- **Comparison of different Carriers:**
  - > critical parameter is depth; as deep as necessary and as flat as possible
- **Comparison of different fillers:**
  - > BSA suitable for most samples
- **Comparison of different cell types:**
  - > suitable for different samples, e.g. HeLa, MDCK; yeast, bacteria, ...



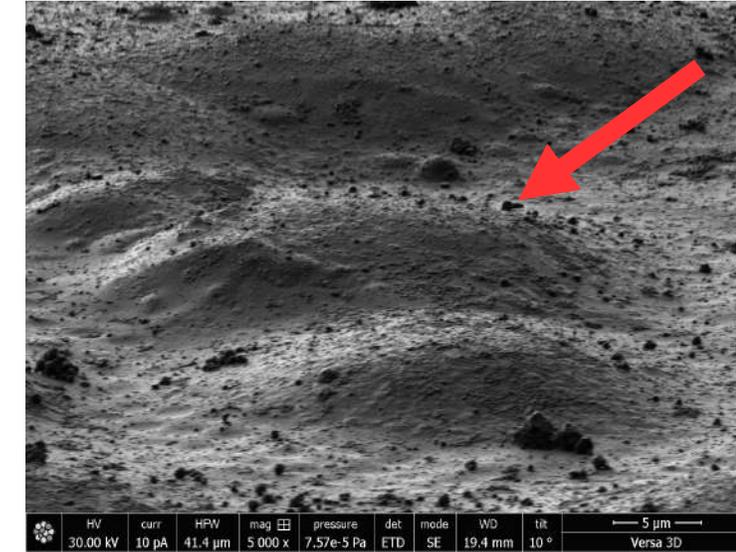
# Project Results: cryo-FIB

- **Complete workflow established including:**
  - > cryo-transfer to FIB
  - > FIB Milling
  - > cryo-transfer to TEM
  - > TEM visualization

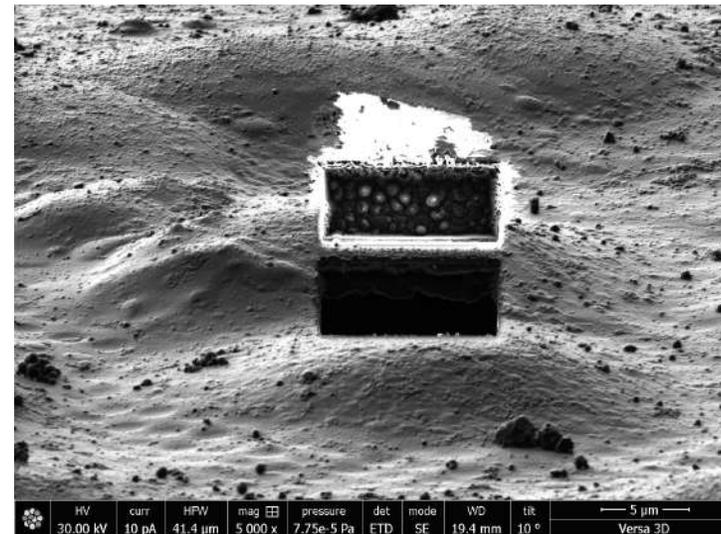
1. Vitrified cells on the TEM grid



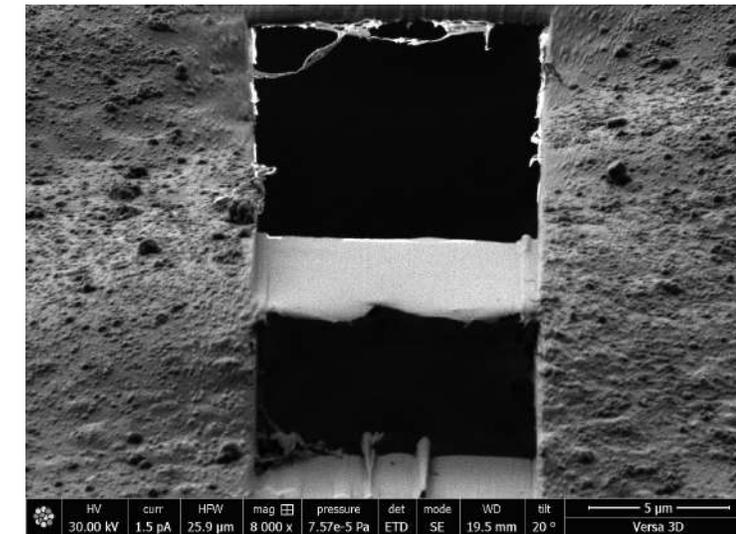
2. Zoom-in to cells selected for lamella preparation



3. Cellular cross-section preparation by Ga<sup>+</sup> beam



4. Final lamella for high-resolution cryo-electron tomography

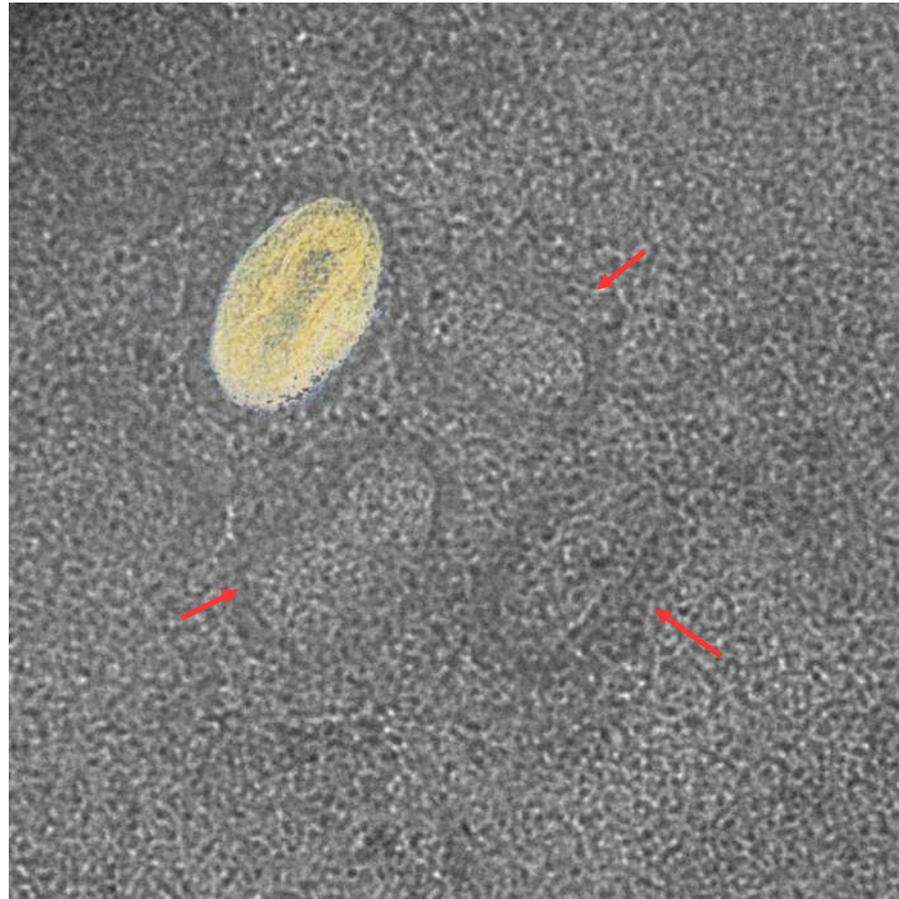


# Project Results: TEM visualization

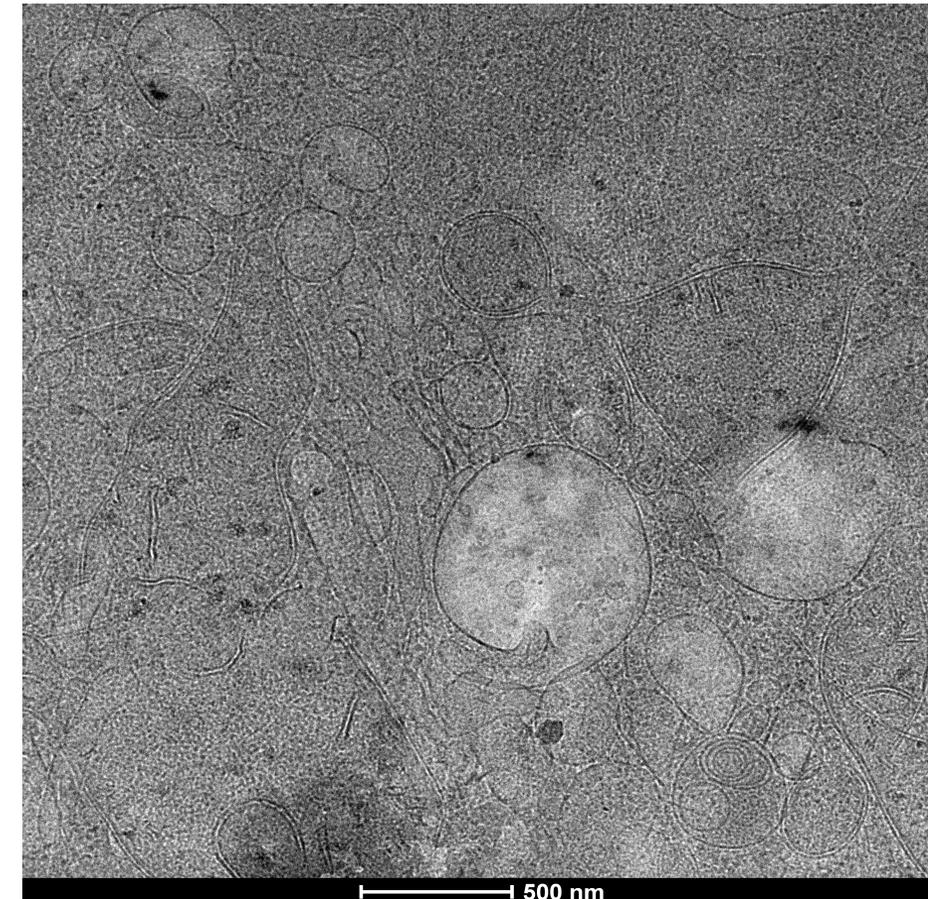


- **Final visualization step:**  
-> transfer to TEM (transmission electron microscope) for 2D or 3D visualization

Vaccinia virus inside A9 cell



HeLa cell



# Project Summary & Outlook



- **Workflow HPF – cryo-FIB – TEM visualization established:**  
**-> ready for open access projects**
- **Potential applications / end-users:**  
this technology allows high resolution insights into large samples as close to the native state as possible today and is highly interesting for several research areas:
  - structural biology
  - developmental biology
  - cell biology
  - pharma industry...