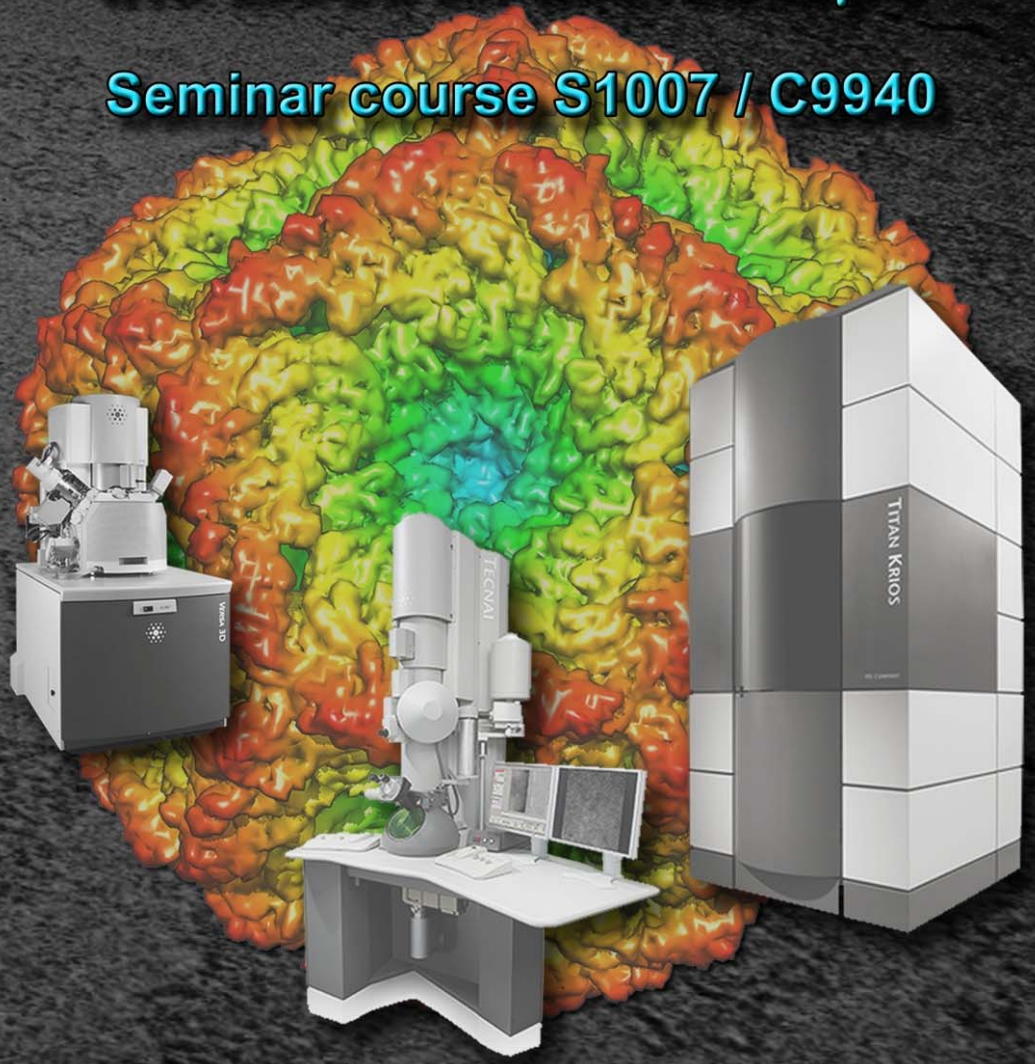
Doing Structural Biology with the Electron Microscope

Seminar course S1007 / C9940



Courses: Mondays 14-16h in A35 / rm 211

Lecturers: Daniel Nemecek & Tapu Shaikh

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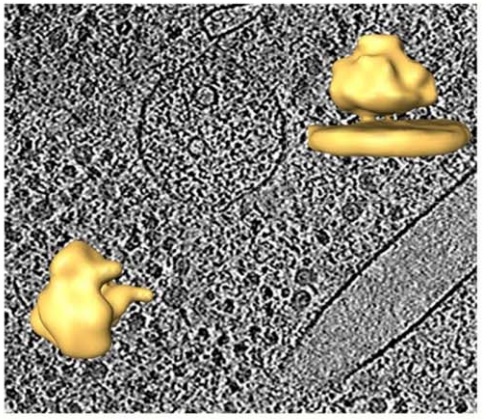
Modern electron microscopy in structural biology on the cellular and molecular level is performed by cryo-electron microscopy (cryo-EM) and cryo-electron tomography (cryo-ET).

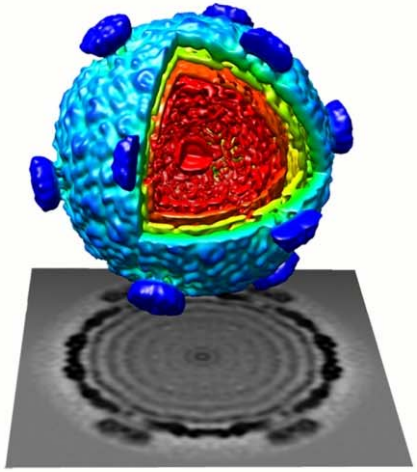
Both methodologies provide information on the cellular and molecular level and are therefore ideal for in-depth structural functional analysis in combination with complementary state of the art biochemical characterisation.

Cryo-EM is applied to study larger macromolecular complexes in vitro as whole functional units, which have been isolated and purified by biochemical methods.

Cryo-ET is the only method to address pleiomorphic structures like cells, organelles and complexes in a native state in situ.

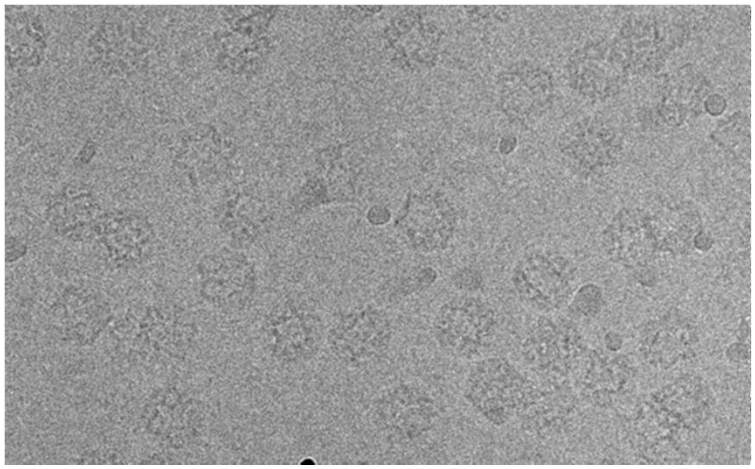
The students will gain knowledge and should understand the principles of structural analysis by transmission electron microscopy, its application in chemistry, biochemistry, structural biology, biophysics and materials science.

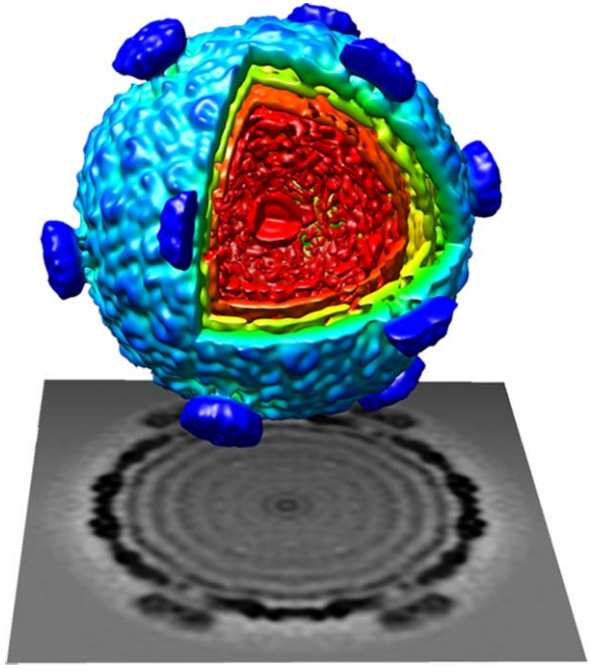




Outline of the CryoEM Course

1. History of cryoEM & facility tour
2. Electron microscope
3. Sample preparation techniques
4. Image analysis I
5. Image analysis II
6. 3D reconstruction
7. Single particle analysis
8. Tomography I
9. Tomography II
10. 3D visualization and segmentation
11. Hybrid methods
12. Practicals: Image analysis
13. Journal club and summary

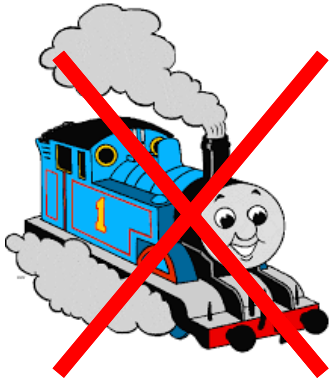




CryoEM Facility: Requirements

Factors influencing performance:

- mechanical or acustic vibrations
- electromagnetic interference (EMI)
- thermal fluctuations



Microscope Room:

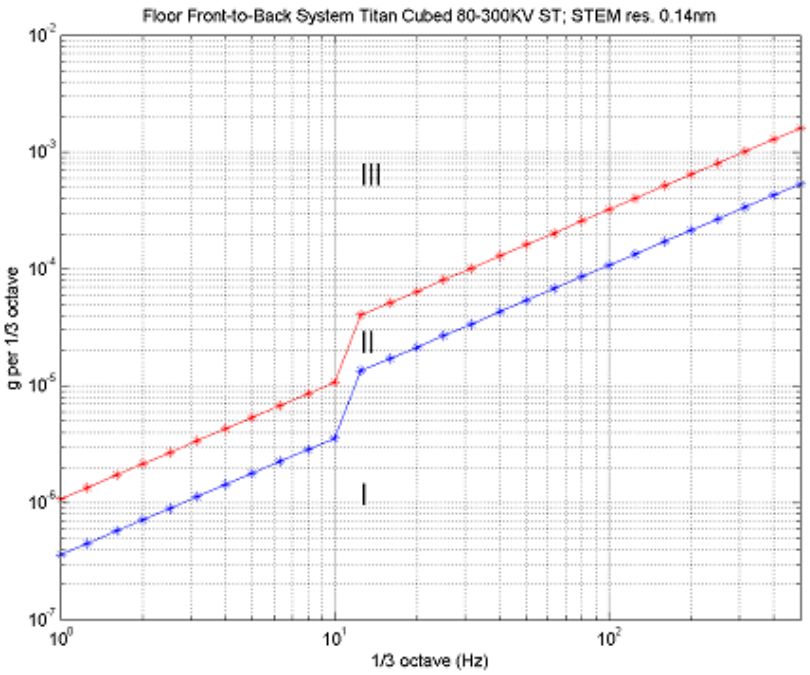
- low level of vibrations and noise
- low level of stray electromagnetic fields
- special requirements for air and heat flow
- cooling chillers located in an adjacent room
- compressed air supply for pneumatics

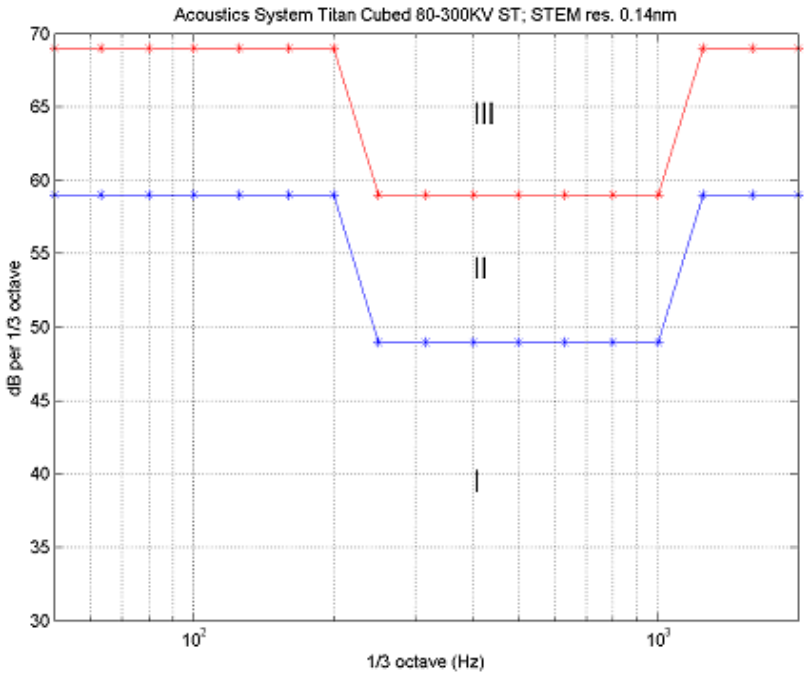
General Safety:

- high voltage and X-ray shielding
- SF_6 and oxygen detectors and exhaust
- precautions for using liquid nitrogen

Vibrations

Specifications: *Entire spectrum* < 70dB *Third-octave bands* < 55 dB





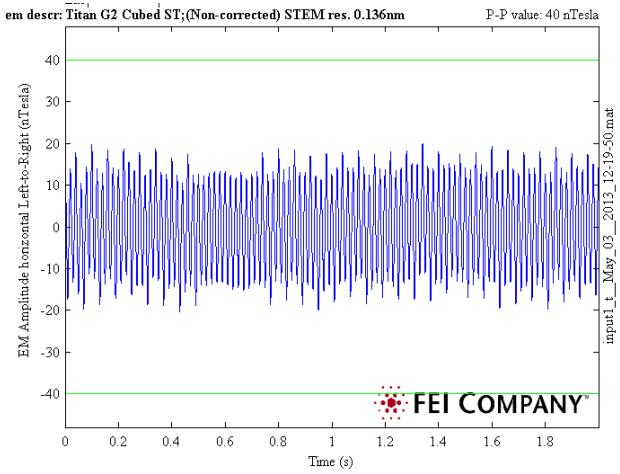
Contra-measures:

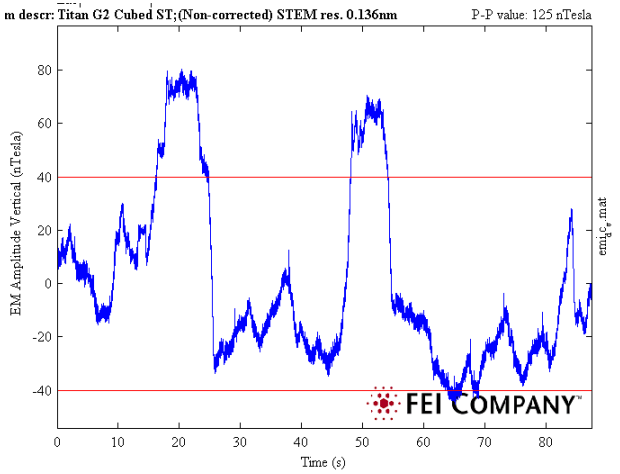
- the microscope room is located in the basement
- the microscope sits on a separated slab of concrete
- installations for passive dumping of vibrations (acoustic absorption panels)

Electromagnetic Fields

Specifications:

Microscope Type	*Horizontal*	*Vertical*
Tecnai 120-200 FEG, TEM	80 nT p-p	95nT p-p
Titan 80-300 FEG, TEM	80nT p-p	80nT p-p
Titan 80-300 FEG, GIF 0.3 eV	50nT p-p	75nT p-p





Contra-measures:

- microscope suite/bunker outside of the main building frame
- passive shielding (Faraday cage) of the microscope room
- active shielding compensating for EMI in real time

Air and Heat Circulation

- Microscope produces significant heat dissipation (~6 kW).
- Dust free, air-conditioned room is strongly recommended.
- The microscope column should not be in the flow of air circulation.
- Cooling units (chillers) should be located in a different room.
- Chemical/oil resistant and antistatic floor covering is needed.

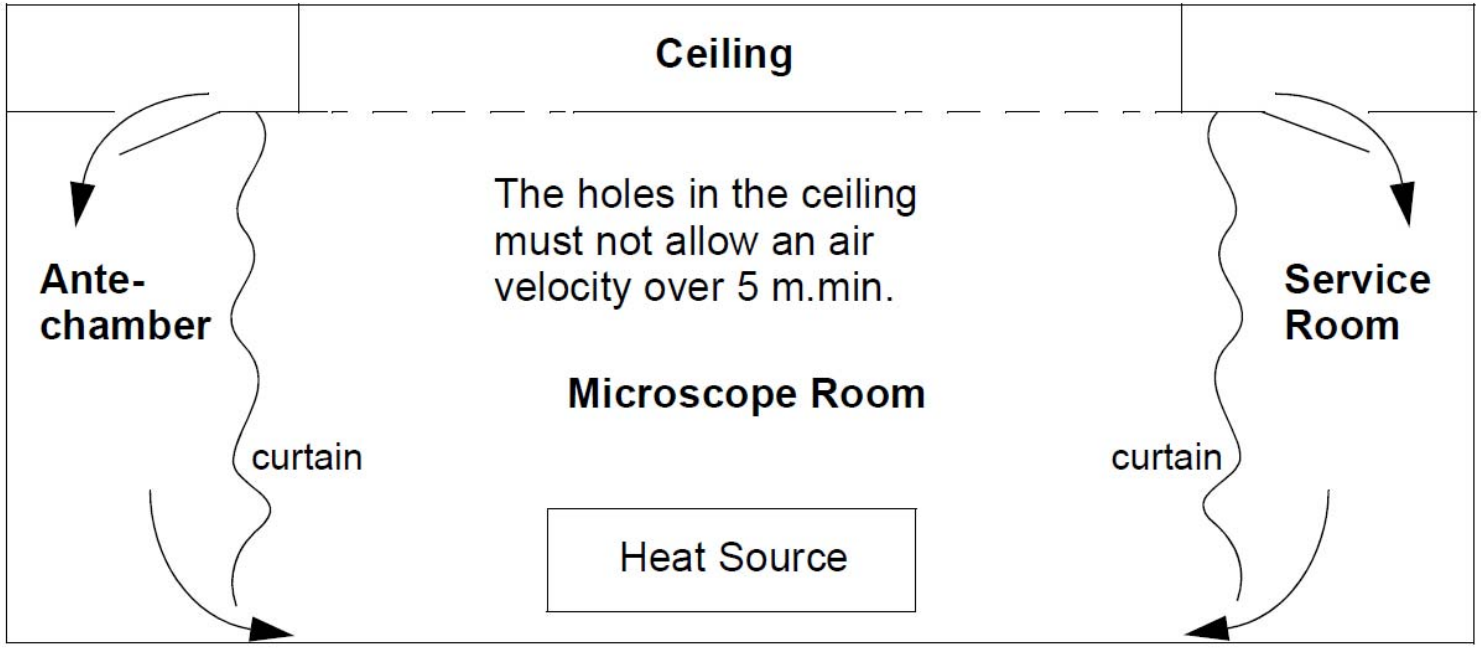
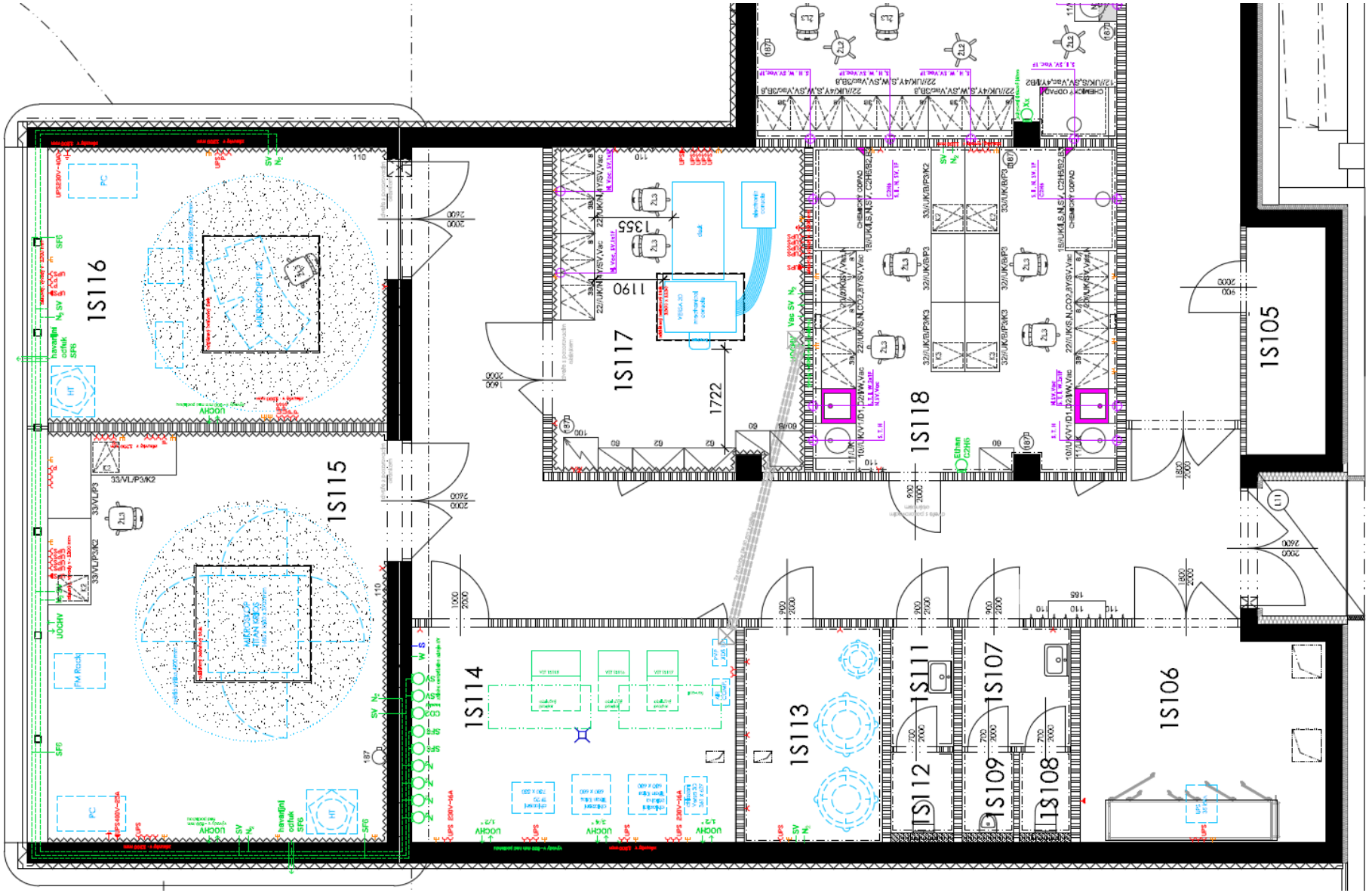


Fig. 3-1: Cross Section of TEM Room - Air Circulation

CryoEM Facility: Plan of the CF in CEITEC



Electron Microscopes

Titan Krios with a direct detector => determination of near-atomic structures

Tecani F20 with a 4k CCD => routine cryoEM to subnanometer resolutions

Versa3D SEM/FIB with cryo transfer chamber => FIB milling of vitrified cells