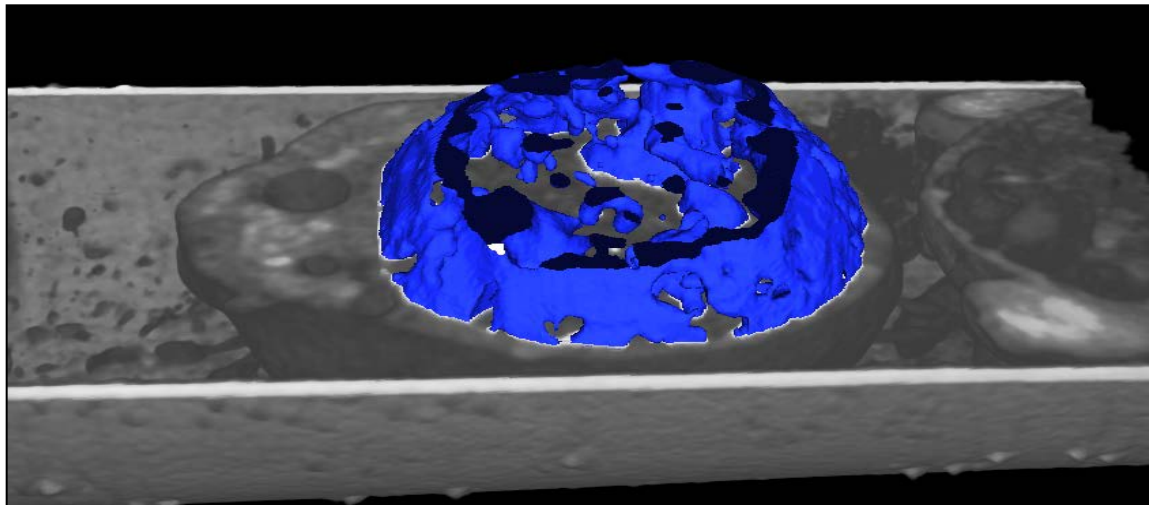


3D Characterization of Chromatin Structure (S65)

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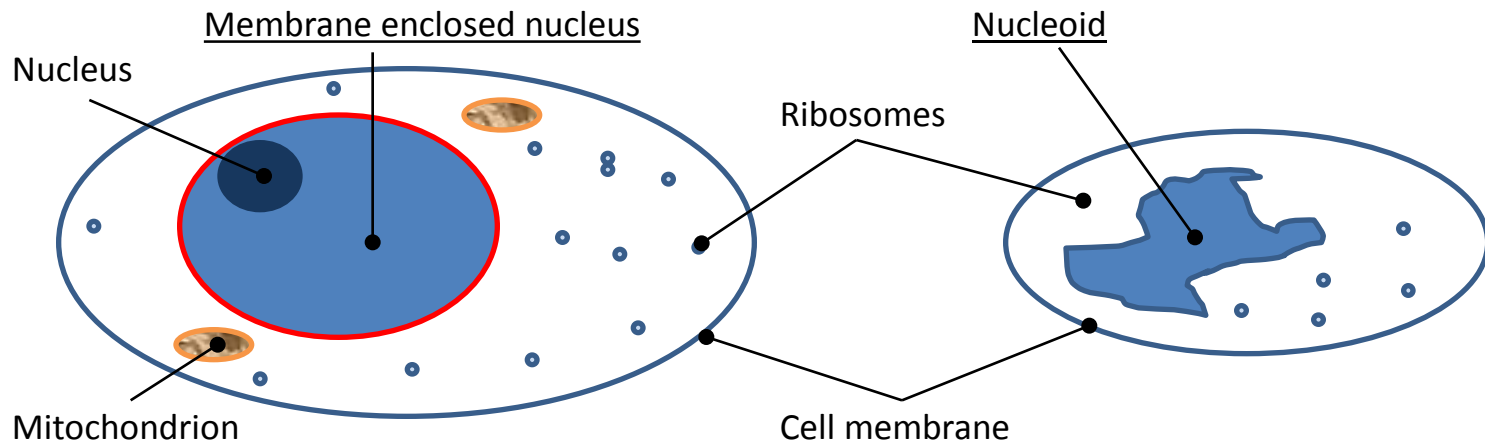
Goals

- Introduce a new 3D imaging technique
Soft X-ray Tomography (SXT)
- Disclose the potential of SXT to reveal
nuclear architecture
- Preliminary results for chromatin structure

Contents

1. Samples
2. Soft X-ray Tomography (SXT)
3. Results

1. Samples

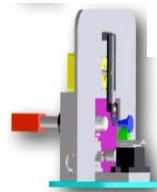
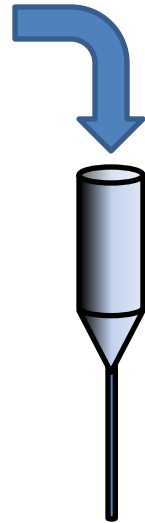
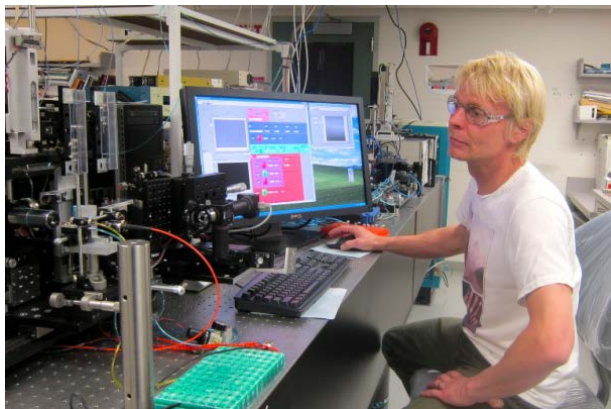
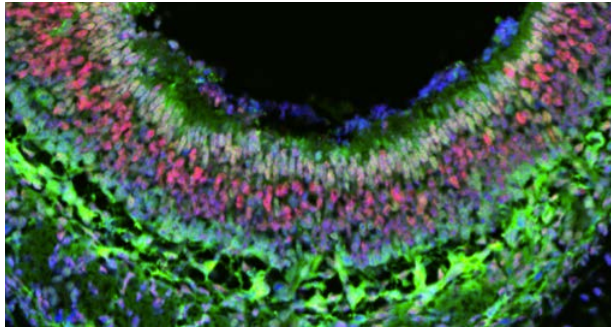


HSV-1 Infected ECFP--H2B B cells

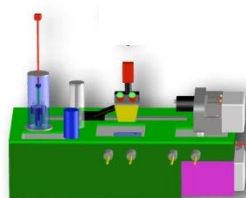
Seven infected and non-infected B-cells were analysed

1. Samples

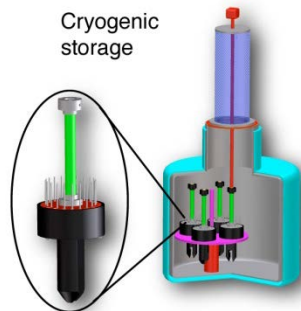
- Grown in culture
- Loaded into capillaries
- Rapidly freezed
- Transferred to microscope



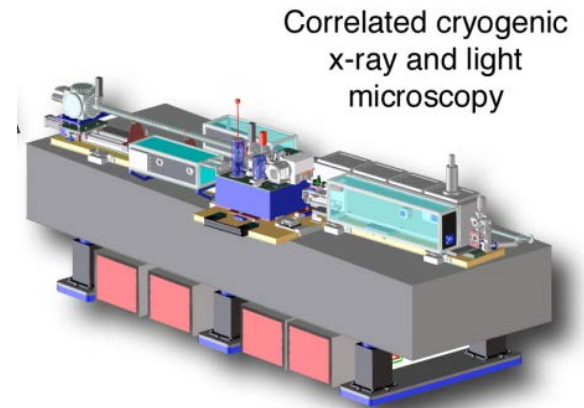
Capillary loader



Plunge freezing cryogenic manipulation and imaging



Cryogenic storage

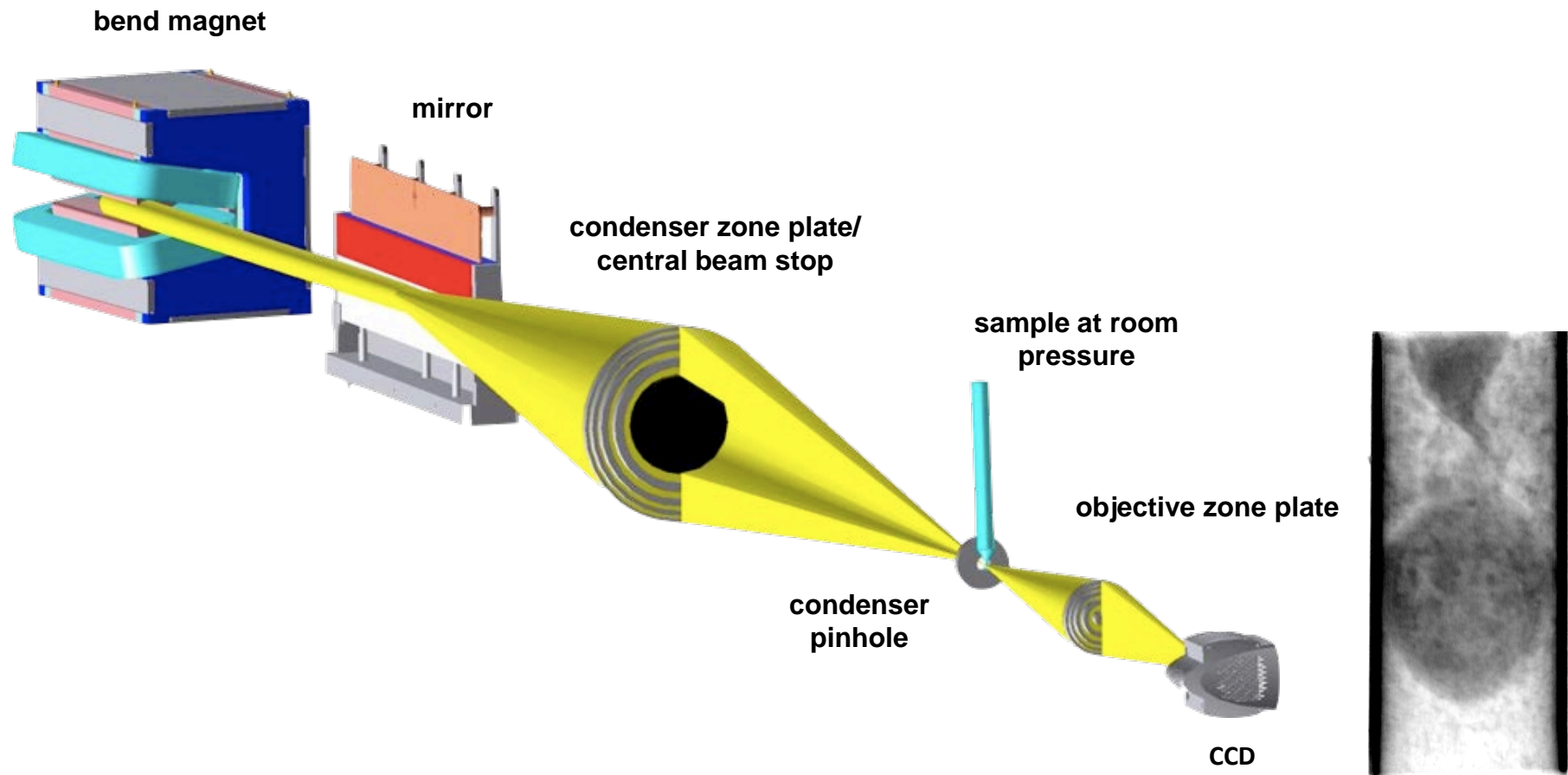


Correlated cryogenic x-ray and light microscopy

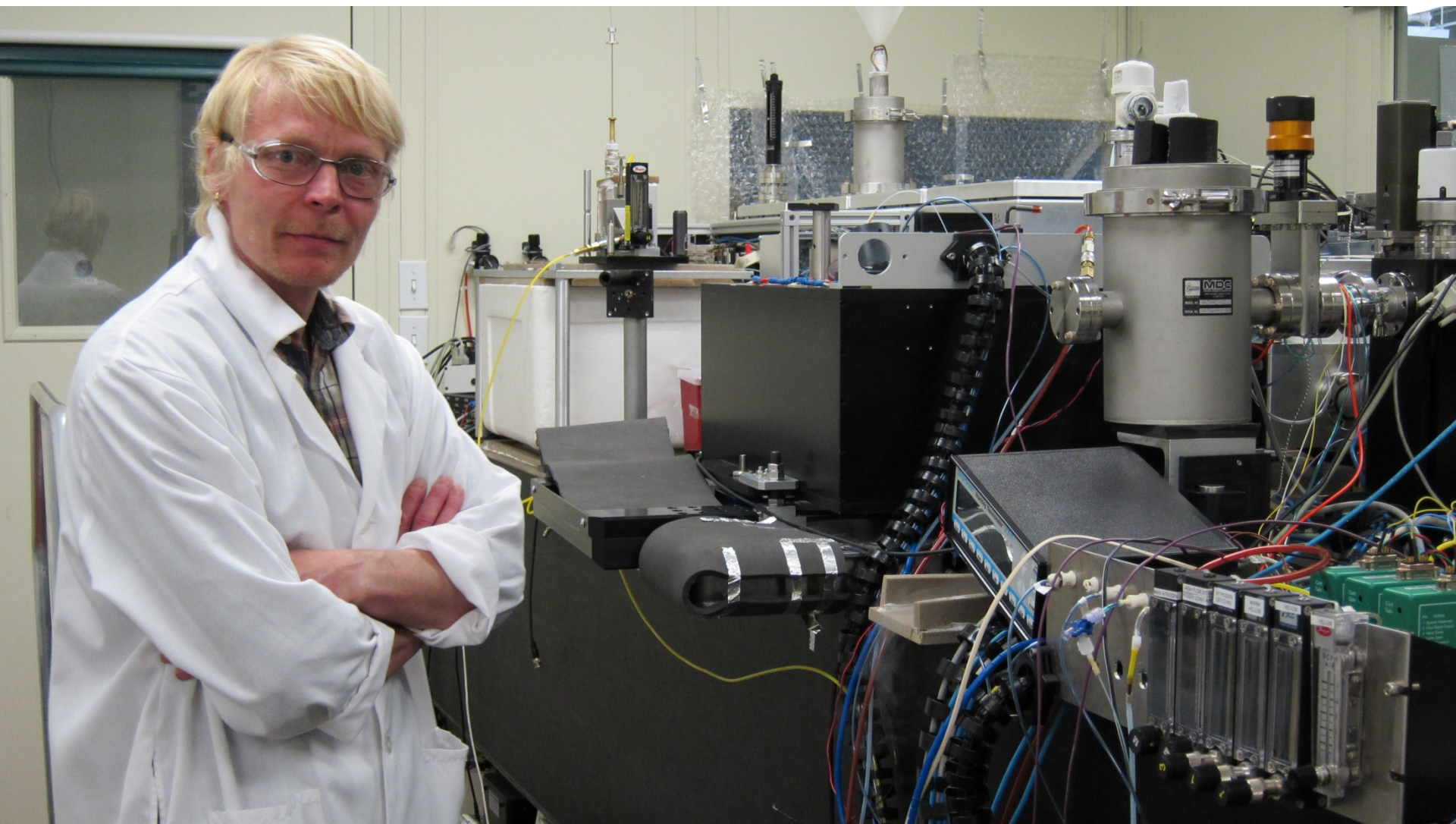
1. Samples

-Whole cells,
that were near
natural hydration state,
were used.

2. Soft X-ray Tomography (SXT)



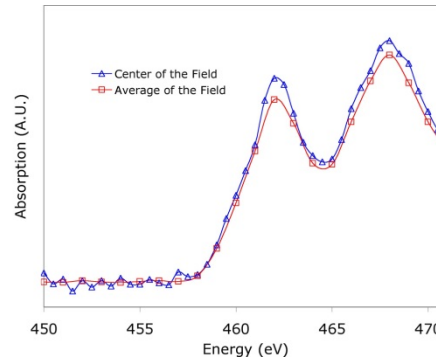
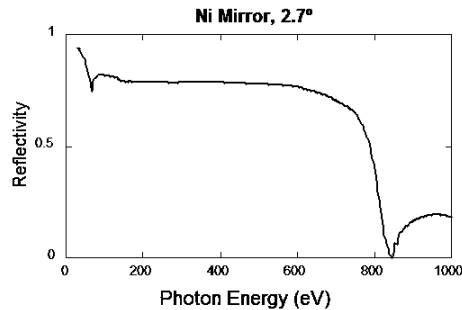
2. Soft X-ray Tomography (SXT)



2. Soft X-ray Tomography (SXT)

Soft X-rays:
 ~ 500 eV, $\lambda \approx 2$ nm

bend magnet



mirror

condenser zone plate/
central beam stop

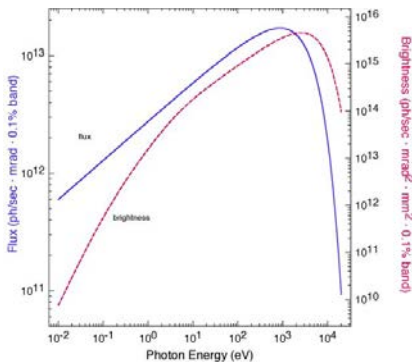
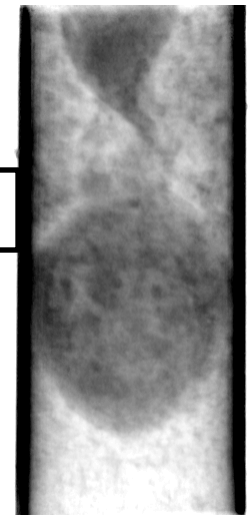
50 nm Resolution

sample at room
pressure

objective zone plate

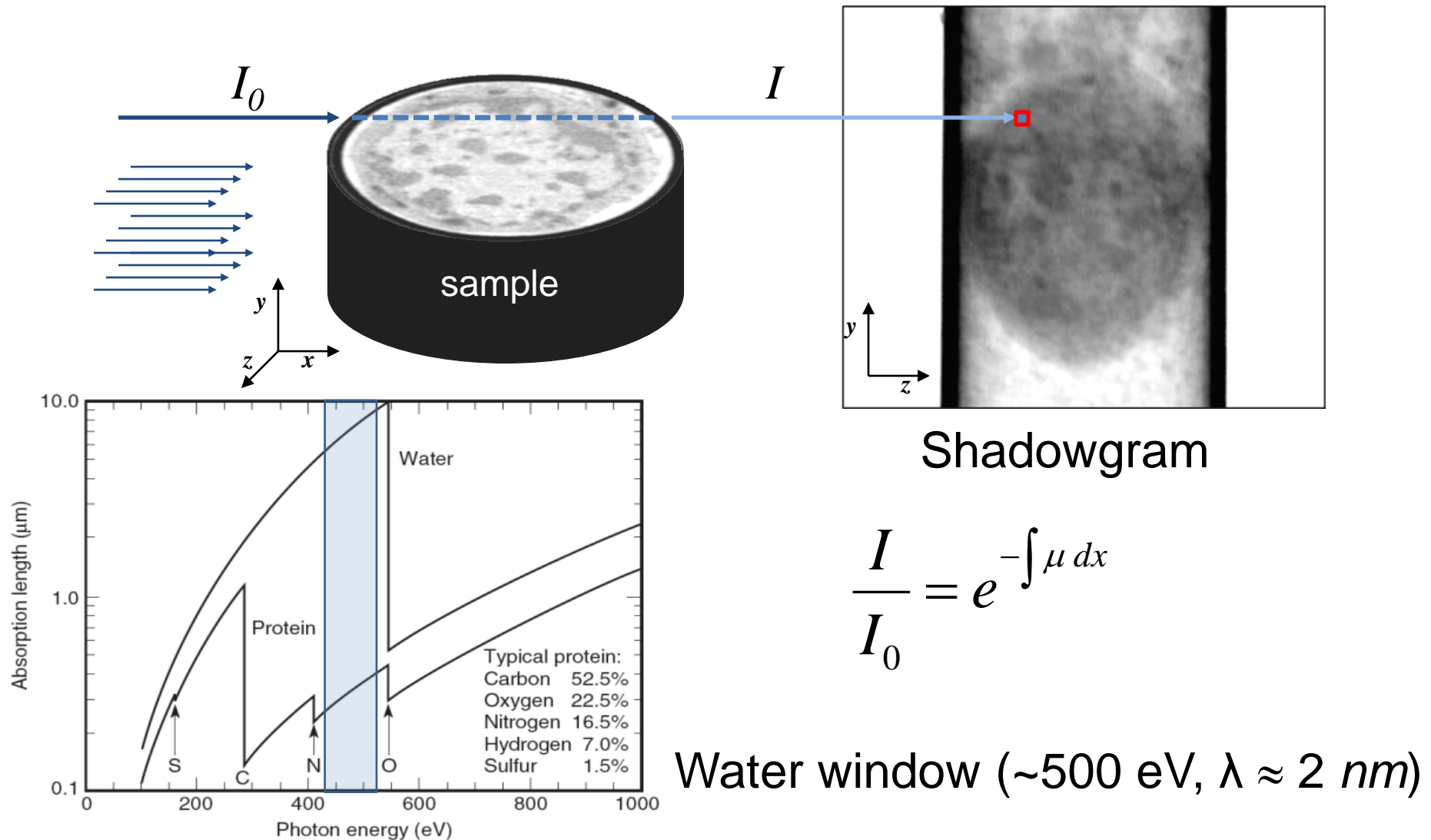
condenser
pinhole

CCD



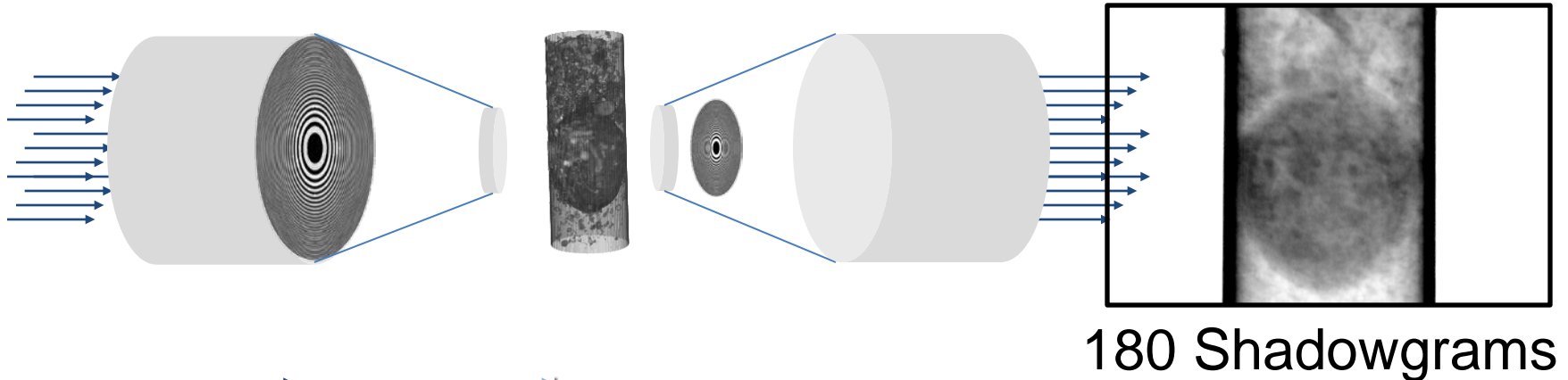
Flux and brightness curves for bend magnet.

2. Soft X-ray Tomography (SXT)



2. Tomography

-Full 180° rotation, with angular steps of 1° .

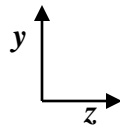


-180 shadowgrams are used to reconstruct three dimensional distribution of Linear Attenuation Coefficients (LAC).

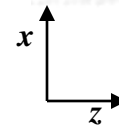
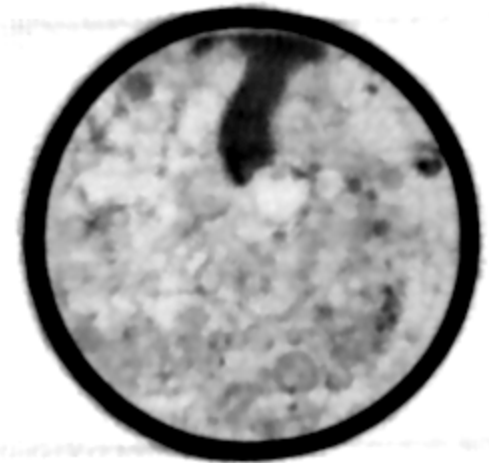
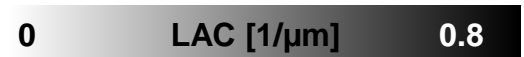


2. Segmentation

Three dimensional
distribution of
Linear Attenuation
Coefficients (LAC).

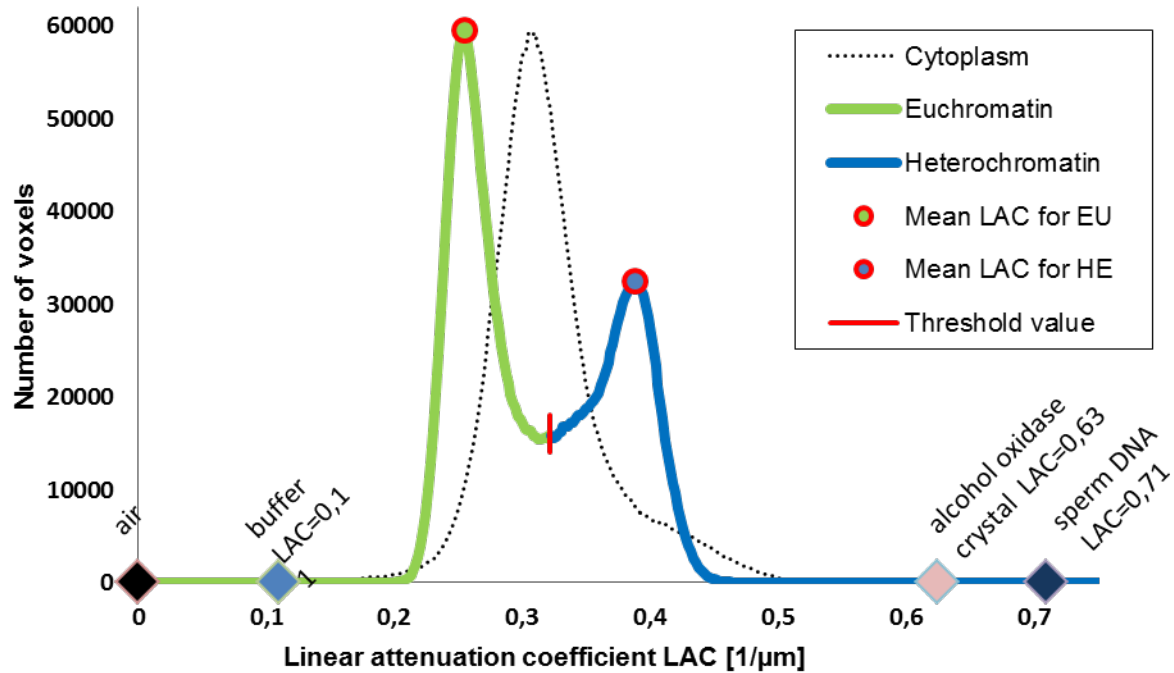


Orthoslices of
Linear Attenuation
Coefficients (LAC).



2. Segmentation

Histogram of Linear Attenuation Coefficient (LAC) for one whole cell

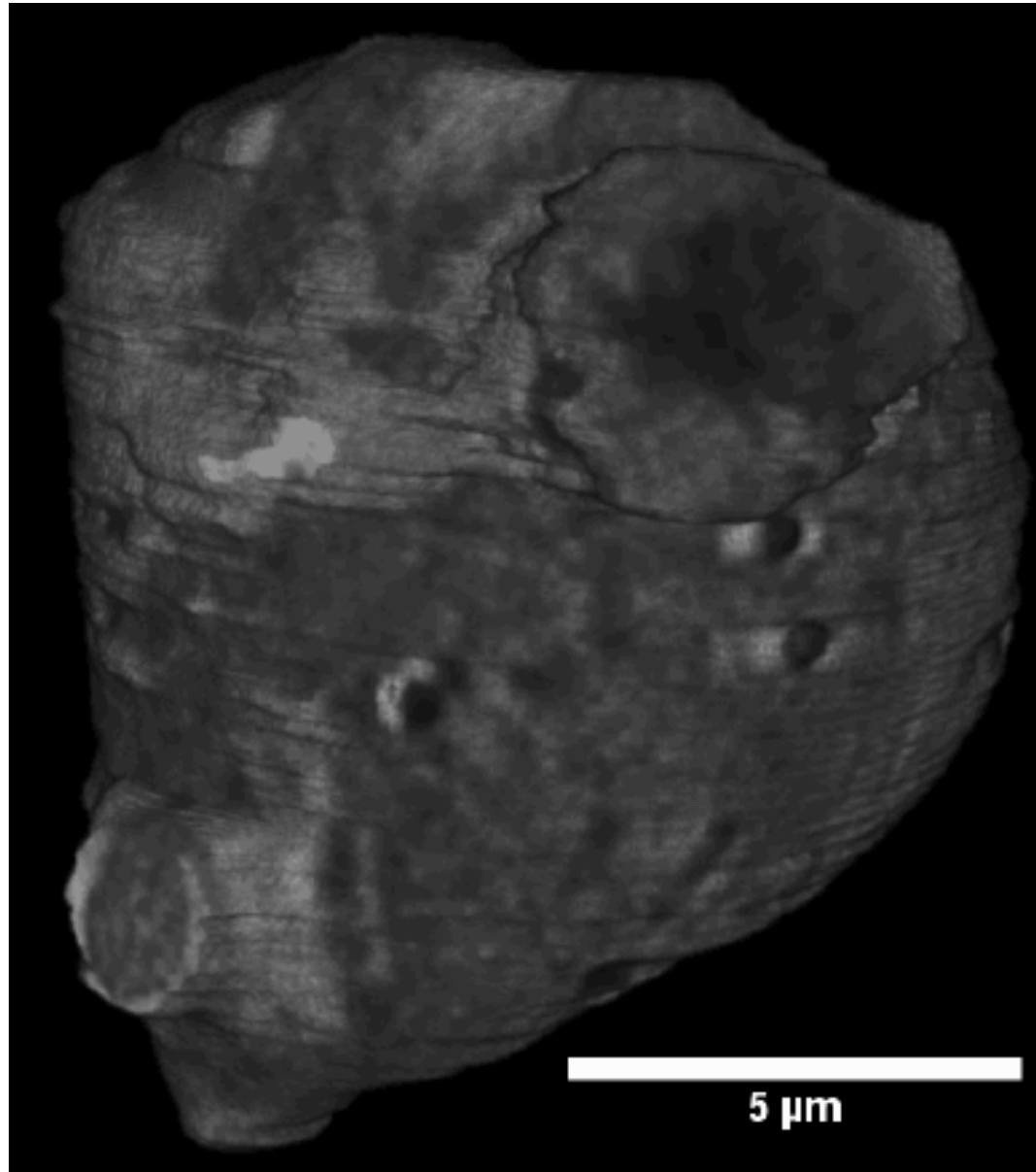


Quantitative analysis:

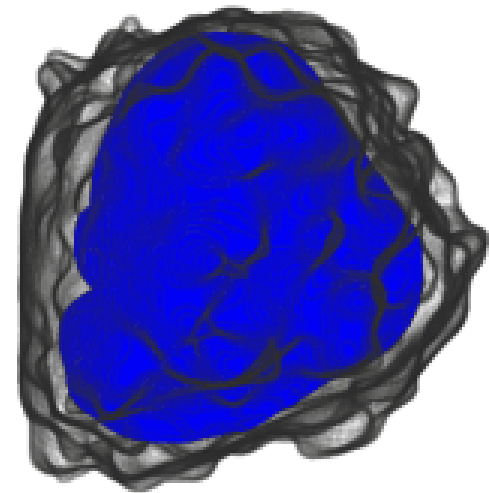
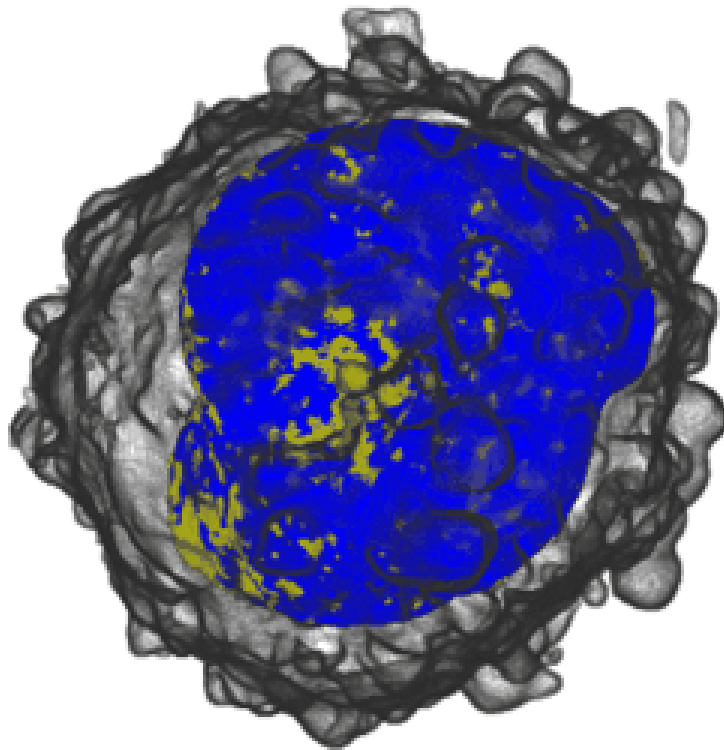
LAC, volumes, surface areas,
structural properties,...

3. Results

- Heterochromatin
- Nucleoplasm with euchromatin
- Nucleoplasm with viral replication compartment



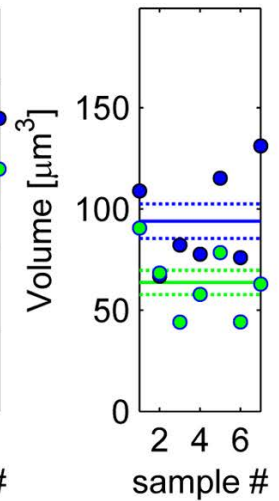
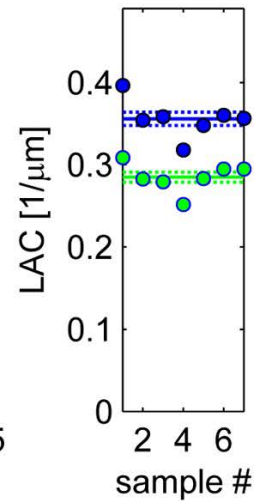
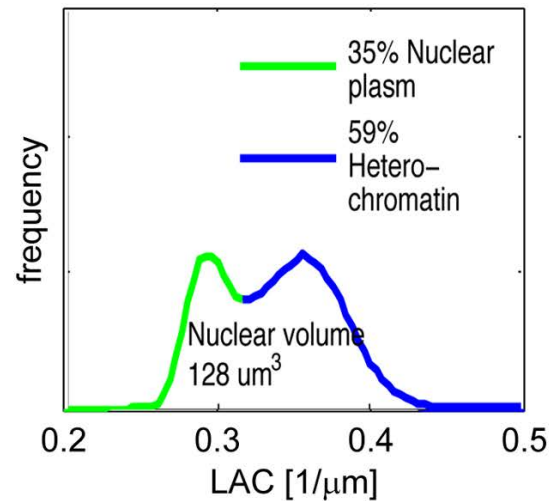
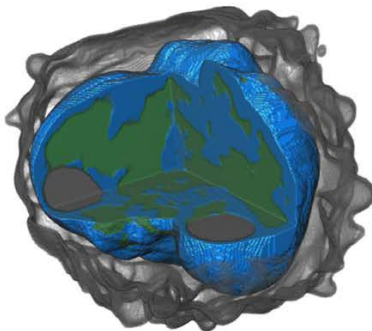
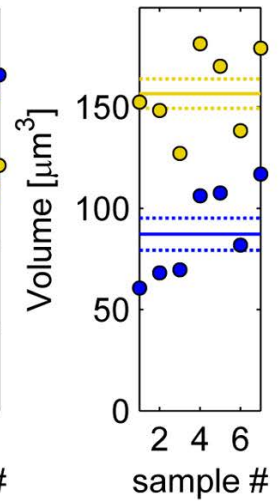
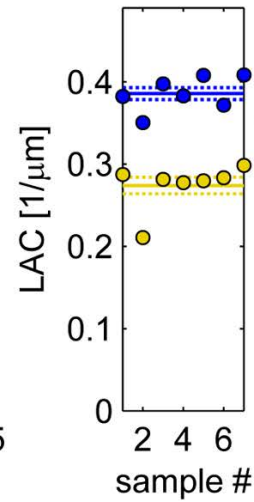
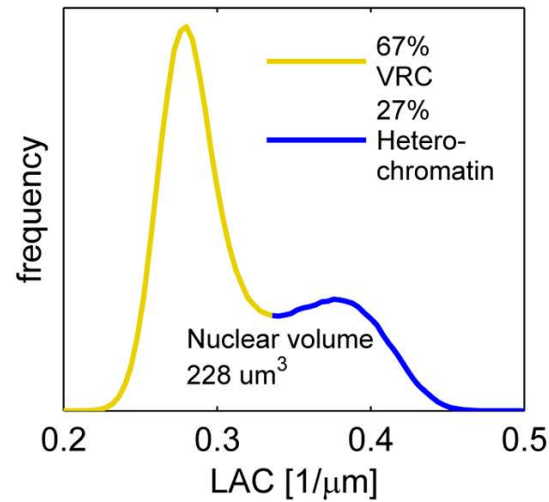
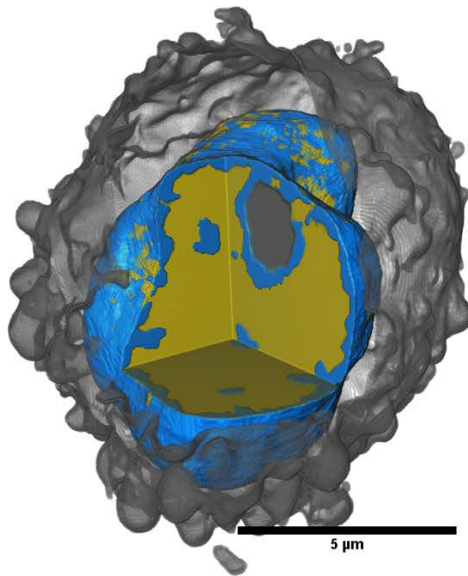
3. Results



5 μm

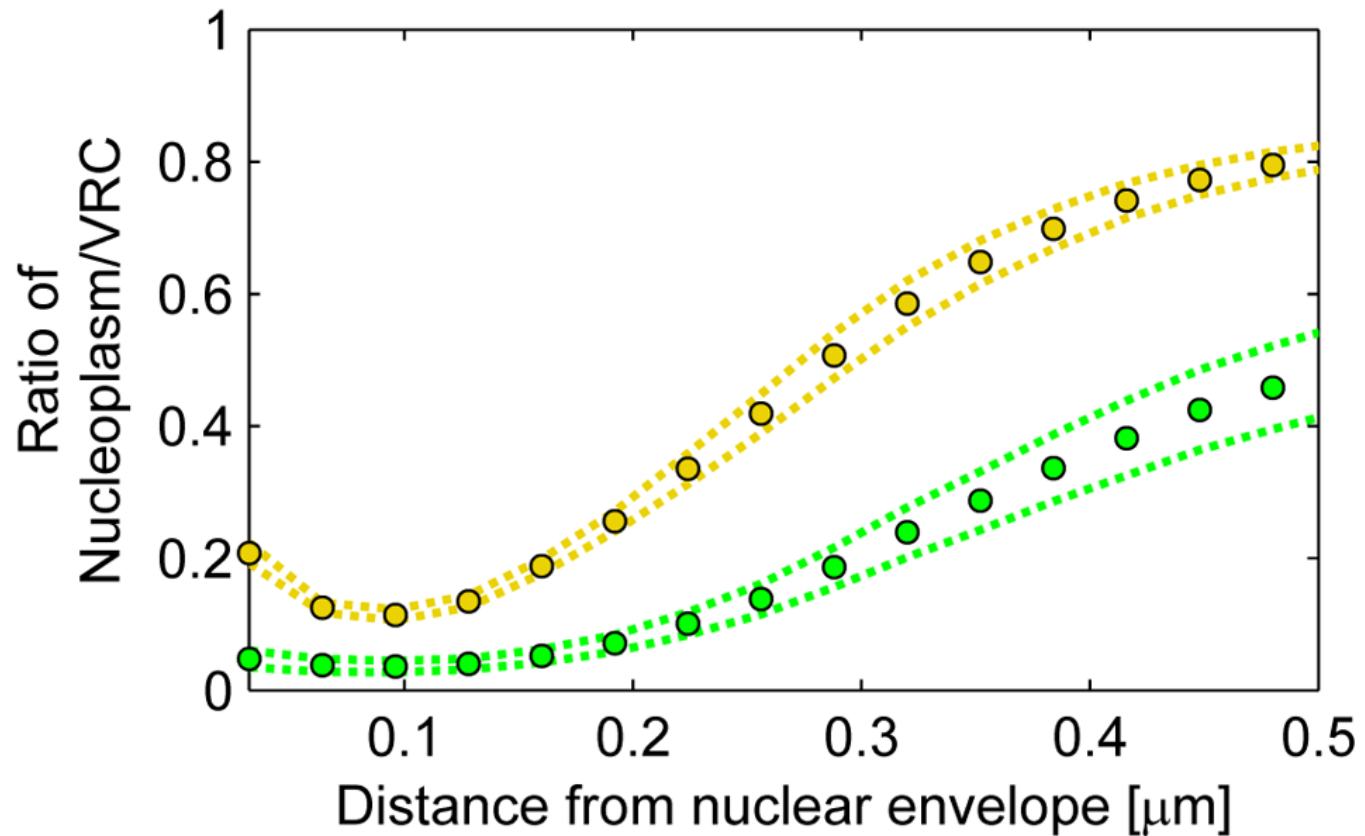
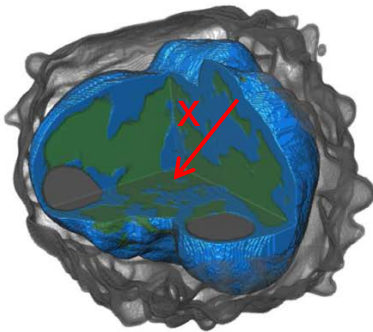
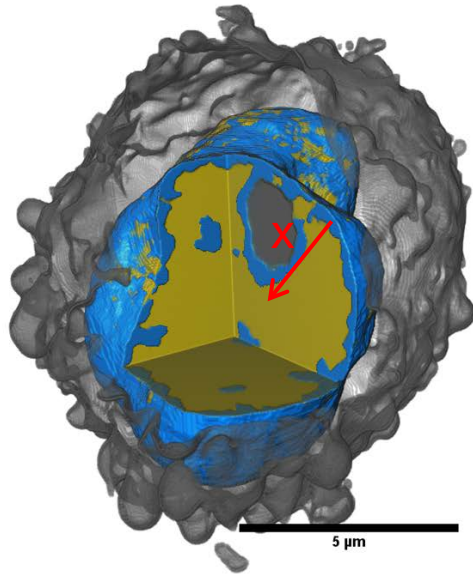
3. Results

Volume and LAC values



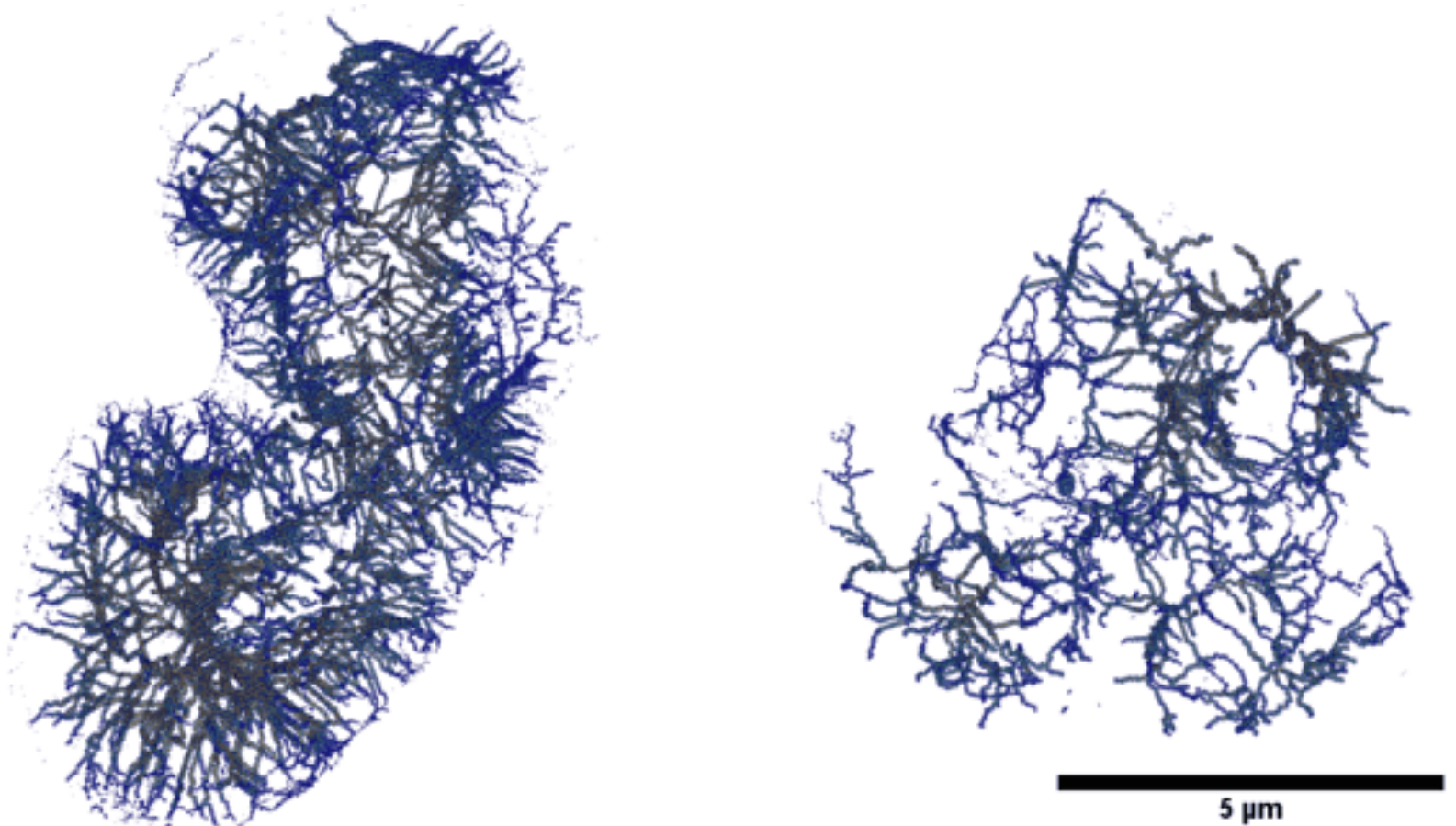
3. Results

HSV-1 infection induces relocation of heterochromatin to the nuclear periphery



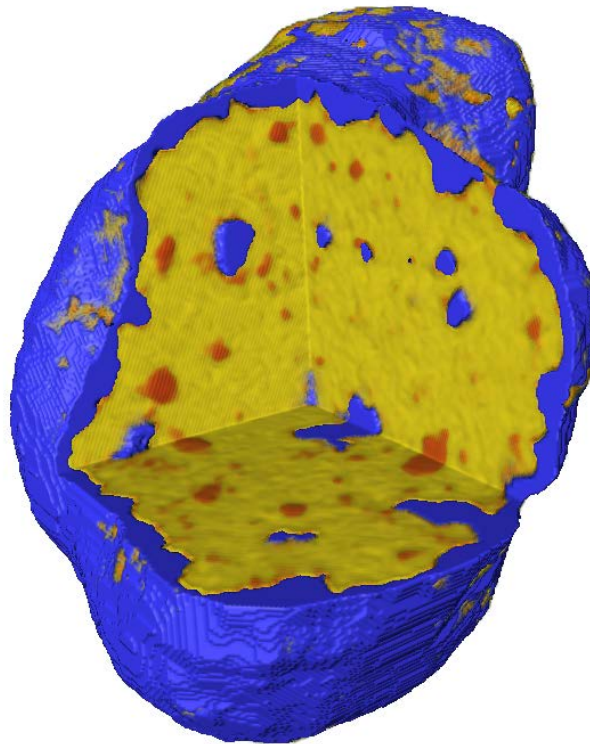
3. Results

Infection establishes NPC-independent channels through the heterochromatin layer



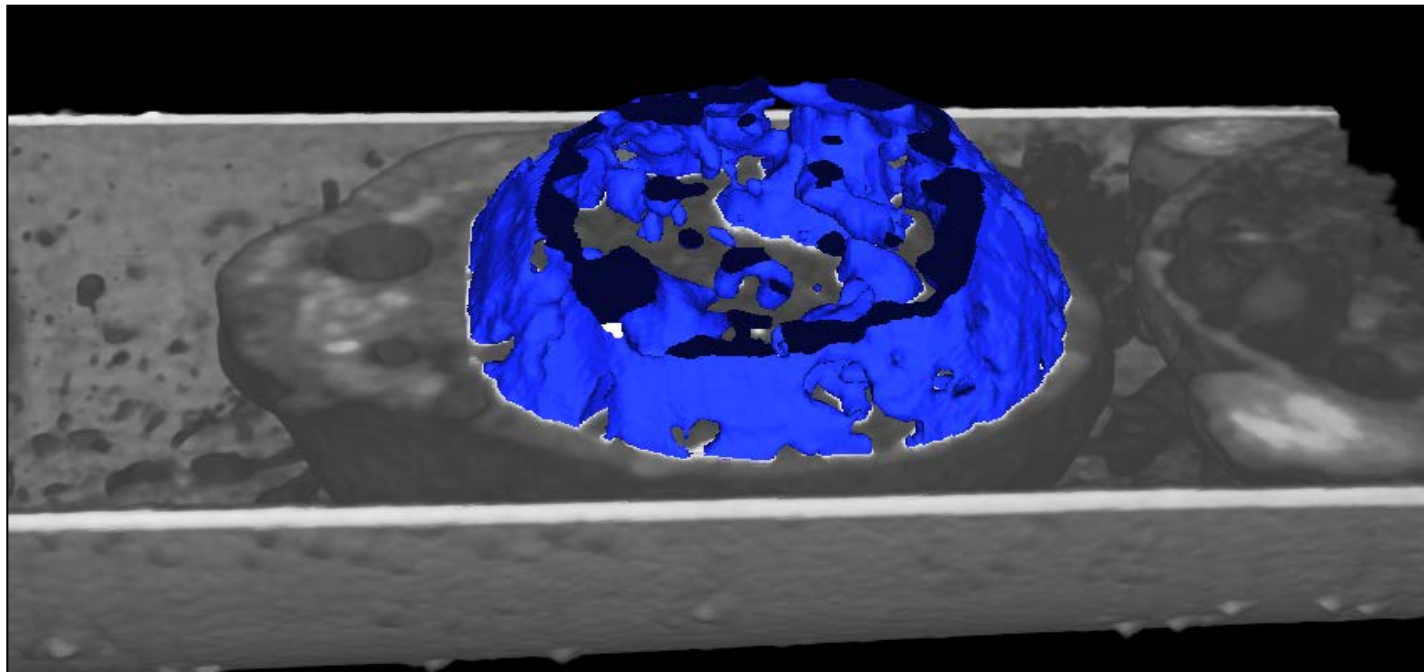
3. Results

Enlarged **nucleoplasm with replication compartment** contains foci and allows high degree of mobility



Conclusions

Soft X-ray Tomography can provide novel understanding of the nuclear structure, which is expected to be useful in basic cell biology and in medical applications related e.g. to cancer research and viral infections.



Thank you for your attention.