Nanoscale Chemical Imaging of Phagocytosis: A Battle for Metals between Host and Microbe

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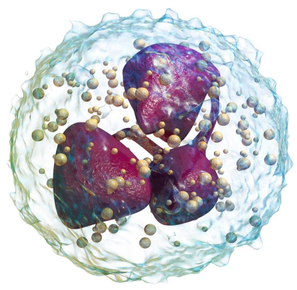
See the video on phagocytosis!

On Metals, Neutrophils and Phagocytosis

Metals are crucial for the survival of living organisms. Since metals are often scarce in biological habitats, organisms had to evolve sophisticated take-up systems in order to survive.

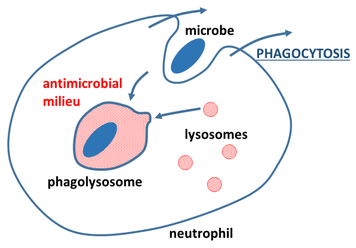
Zn^{2+} Mn^{2+} Ca^{2+}



Neutrophils (PMNs or polymorphonuclear leukocytes) are the most abundant type of phagocytes which patrol the blood stream and can quickly migrate to an emerging infection site

Phagocytosis is a major self defense mechanism discovered by Élie Metchnikoff in 1882 in which neutrophils engulf microbes. The enclosed vesicle or phagosome fuses with granules releasing their lethal content:

- Reactive oxygen species (ROS)
- Nitric oxide
- Antimicrobial proteins/peptides

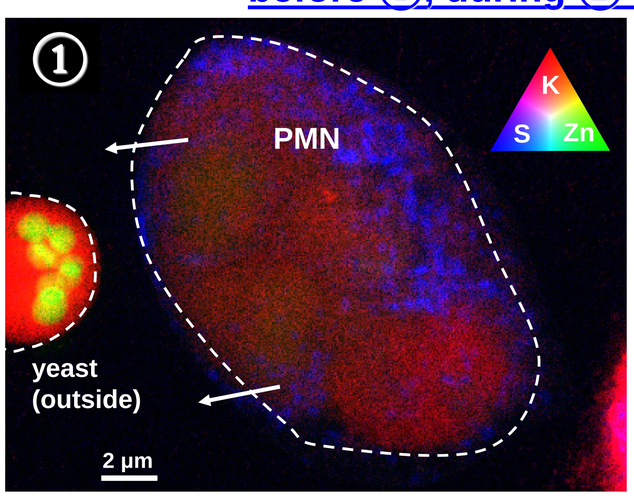


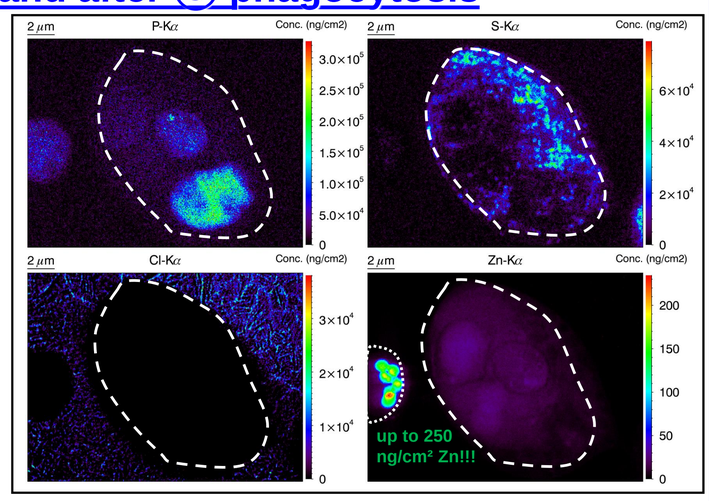
⇨ During phagocytosis of microbes by neutrophils, it is unclear which metals are retained or accumulated in the phagosome!

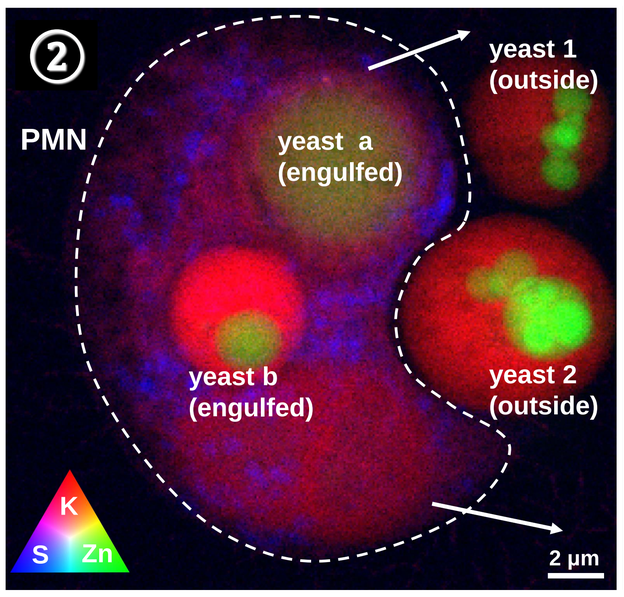
Aim of our study:

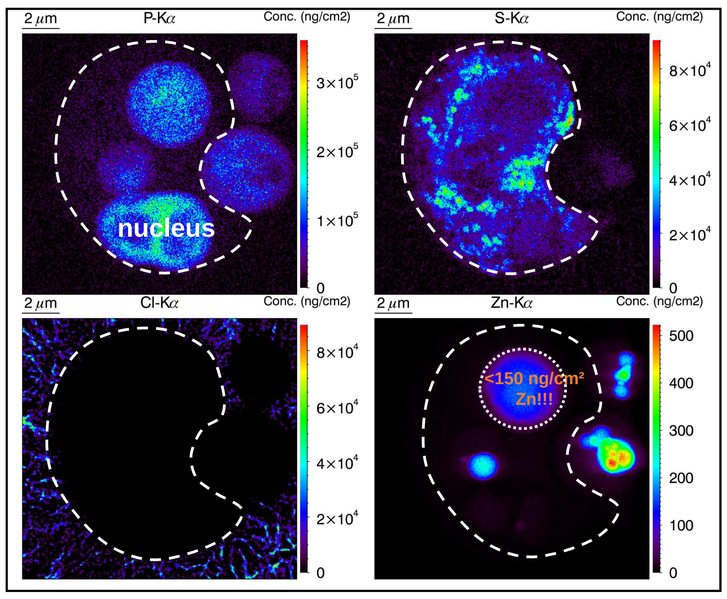
1. Perform nanochemical imaging upon cryofrozen neutrophils phagocytosing *S. cerevisae* (baker's yeast).
2. Quantitative comparison of the chemical content of yeast a) outside the PMNs and b) inside the phagosome of PMNs.
3. Confirm whether yeast cells are effectively within the neutrophils and not on top/below (using 3D phase contrast imaging).
4. Compare phagocytosis between opsonized and non-opsonized yeast.

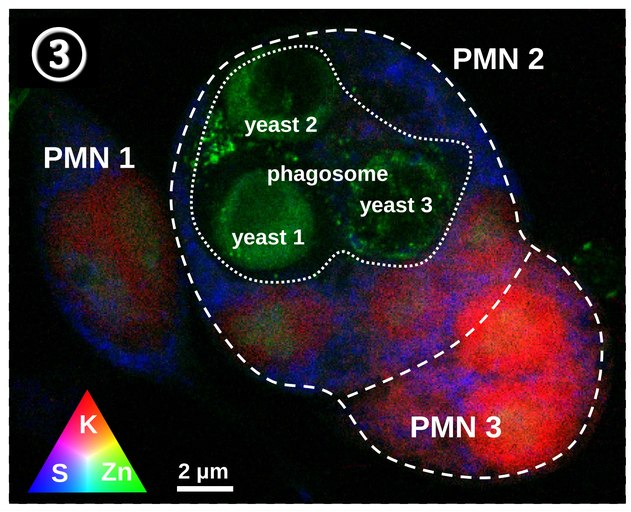
Experimental Results: nanochemical imaging before ①, during ② and after ③ phagocytosis

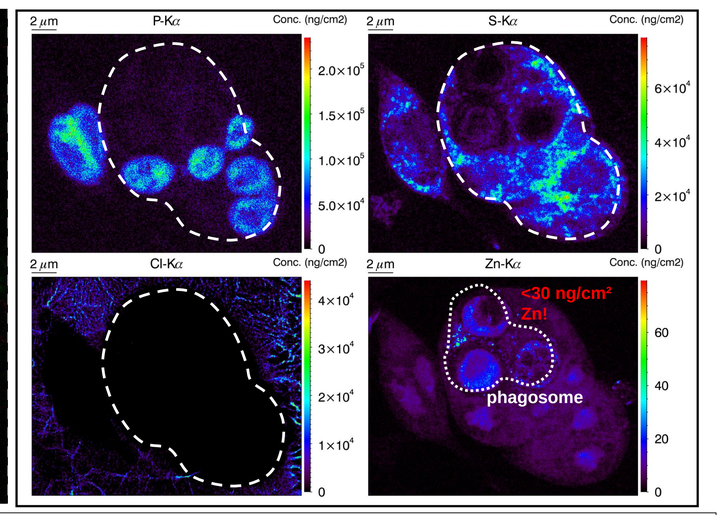






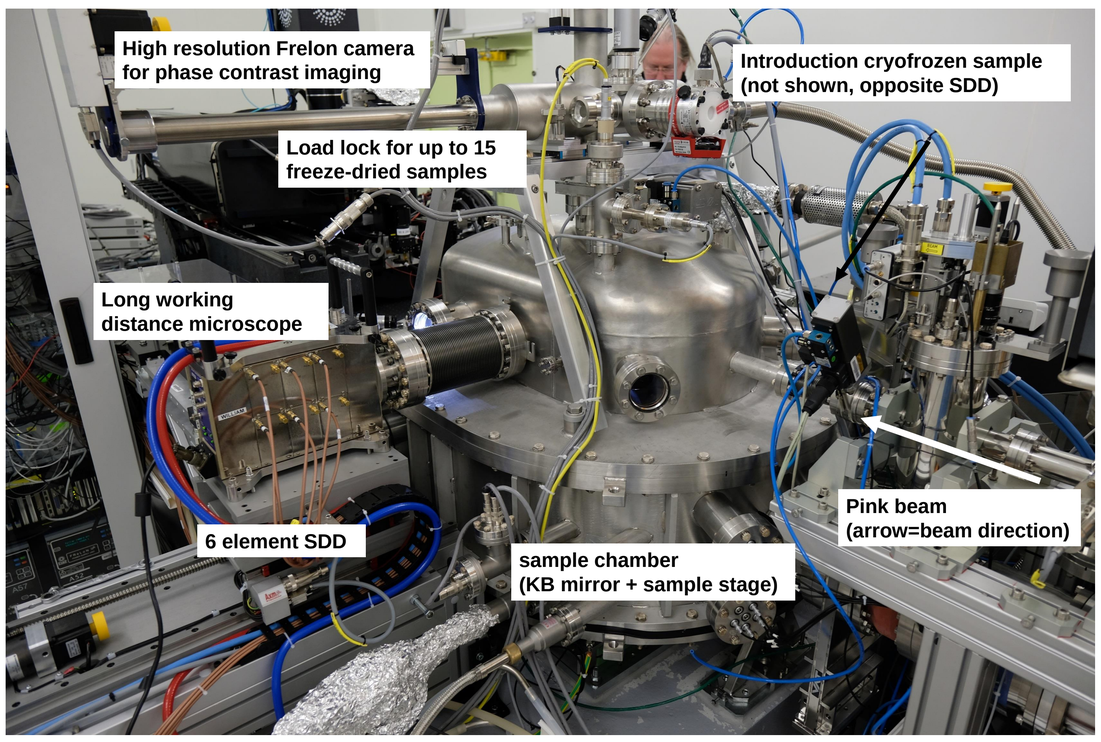




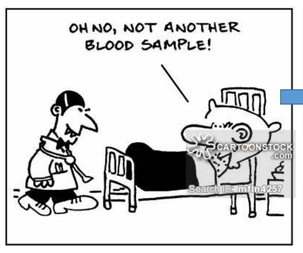


Normalization: all elemental maps were normalized to 200mA ESRF ring current and corrected for dead time (mean value over entire map approx. 10% at high dose: $2x10^{11}$ photons/s and 50 µm secondary source size). **Conversion to areal concentrations** (expressed in ng/cm^2) was done by using the Fundamental Parameter equation and by measuring a thin flake of NIST SRM 1577C (bovine liver). **Self-absorption** effects within the SRM and from the ice layer covering the cells were corrected for as well. **Ice thickness** of the samples (14.9, 10.9 and 9.4 µm resp.) was calculated from the K-$K_\alpha K_\beta$ ratio. When assuming the cells form a uniform layer of 10 µm thickness, areal concentrations in ng/cm^2 can be approximated by weight fraction in ppm (parts-per-million). **Background subtraction** was performed by subtracting the mean areal concentration from a manually defined background cluster, i.e. the area outside the cells. **Limits of detection (LODs)** in scanning mode (50 ms dwell time) for **Zn** (including 10 µm ice layer): **430 ppb, 6.9 ng/cm^2, 62 zg (10^{-21}), 580 atoms or 6.6 µM**. Also elemental distributions of Ca, Fe and Br were obtained; Mn, Ni and Cu were below LOD in scanning mode.

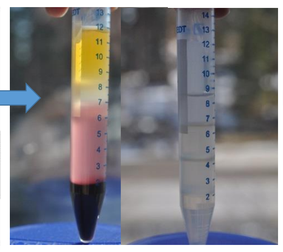
Experimental set-up: ID16NI 'nano-imaging' beamline



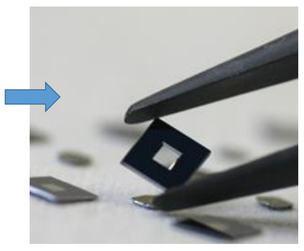
Sample preparation and cryogenic sample chain



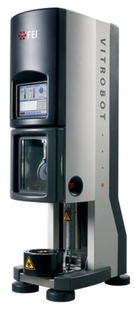
Fresh blood + serum from healthy human donors



PMN isolation with 1:1 blood - histopaque 1119 and Percoll gradient

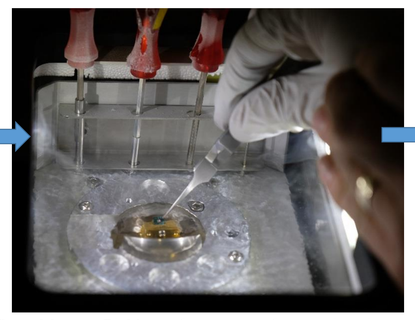


1) seeding on Si_3N_4 wafers
2) infection with S. cerevisae
3) 1-3h incubation

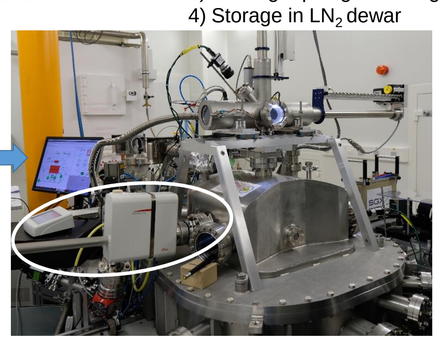


1) Transfer to EM facility
2) Wash in 0.4M NH_4HCO_2
3) Blotting + plunge freezing
4) Storage in LN_2 dewar

Transport samples in dryshipper



Mount Si_3N_4 wafer + transfer to shuttle



Introduction into ID16NI sample chamber

Conclusions and Outlook:

- **High flux** (10^{11} photons/s), **hard X-ray** (17 keV) **nanobeam** (50 nm) with **cryogenic sample environment** (-150°C) provided at ID16NI 'Nano-Imaging' endstation is unique on a world scale and enables **trace level elemental nano-imaging upon cryofrozen cells *close to the native state.***
- After phagocytosis by neutrophils, (opsonized) *S. cerevisae* is **deprived from zinc**!
- No clear phagocytosis was observed for **non-opsonized** *S. cerevisae*!
- Improvements on **iron** detection limits are expected after installation of a dual detector configuration.
- A **significant amount of data (500 GB)** was collected on dozens of PMNs and *S. cerevisae* under different infection conditions, including 2D/3D phase contrast imaging. Spectral fitting is completed; data normalization, cluster analysis and quantification is ongoing.

References:

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