ESRF	**Experiment title:** Cellular localization of iron in whole cell models of human disease using synchrotron radiation x-ray microfluorescence.	**Experiment number**: LS-1839
Beamline: ID-21	**Date of experiment**: from: May 21, 2001 to: May 25, 2001	**Date of report**: August 1st, 2001
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Superoxide dismutase (SOD) is present in all aerobic organisms and plays a protective role against the toxicity of reactive oxygen species. Superoxide anion $O_2^{\cdot -}$, a toxic derivate of O_2, is scavenged by SOD in cells. The importance of this enzyme is highlighted by the recent finding that mutations of SOD cause amyotrophic lateral sclerosis. In this debilitating neurological disorder, motor neurons in the brain and spinal cord degenerate. It has been suggested that $O_2^{\cdot -}$ could release iron from iron-sulfur proteins leading to oxidative damage through redox cycling. Moreover, yeast cells lacking SOD show altered iron homeostasis with increased iron concentration in SOD deleted mutants.

The aim of this experiment was to determine the cellular distribution of iron in *Saccharomyces cerevisiae* wild type cells (strain EG103), and mutant cells lacking copper-zinc SOD (EG118), manganese SOD (EG110), or both (double mutants EG133). In addition the oxidation state of iron in these strains would be determined using XANES (x-ray absorption near edge spectroscopy).

A new gold zone plate (TASC-INFM, Trieste) of 69.9 µm diameter and 200 nm outer zone width optimized for 7 keV was used in the ID-21 scanning x-ray microscope. A 25 µm tungsten wire was used as central stop. At 7.2 keV the focused beam was as small as 0.5 x 0.5 μm^2 in the focal plane. This beam size was compatible with yeast cell dimensions, 5 µm diameter in average, enabling element mapping. However, the photon flux was too limited to allow the detection of trace elements such as iron. Only some major elements S, Cl, and K could be measured and mapped (Fig. 1). As expected, potassium and sulfur distributions, two highly concentrated elements in the intracellular space, depict the cellular shape and some ultrastructures can be recognized such as the nucleus. On the contrary, chlorine is homogeneously distributed throughout the scanned area as this element is essentially extracellular. Single cell element mapping was performed on EG103, EG118, and EG110

strains giving similar results. Using x-ray fluorescence with a non focused beam, it was estimated from Fe/K ratios that an increase of a factor of 30 in incident photon flux would be necessary to perform iron mapping in yeat cells at 0.5 µm resolution. The experimental setup could be improved in a near future in this purpose. First, a central stop of smaller size adapted to the new zone plate will be produced, secondly, multilayers could be designed and used in place of the Si(1,1,1) crystals in the monochromator. Overall the gain in flux should be about two orders of magnitude.

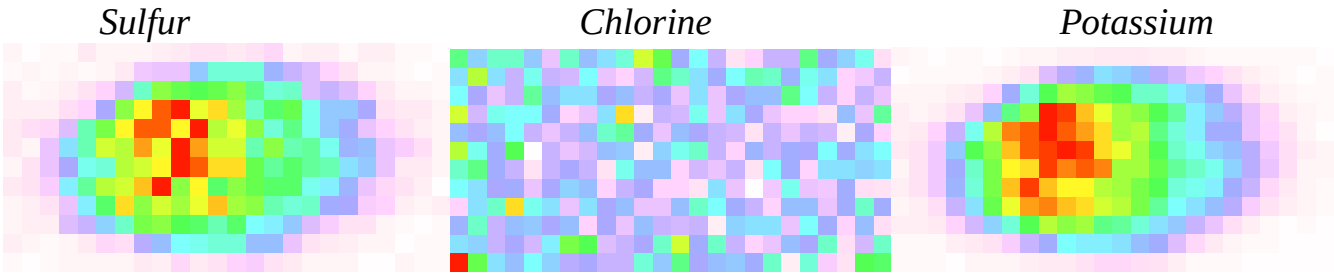

Figure 1. Element distributions in a single cell EG103 (wild type). Scan size = 4 x 7 µm^2.

XANES experiments were performed using a Si(2,2,0) crystal monochromator on bulk samples with a large beam (200 x 200 µm^2) around iron absorption edge. Iron is presumably present as a mixture of Fe(II) and Fe(III) as determined by the centroid position of the pre-edge peak 7.1130 keV, and in the same valence ratio for EG103, EG118, and EG133. However, spectra become slightly differents above the absorption edge meaning that iron local environment is different in EG103 and EG133 cells (Fig. 2.). This demonstrates the feasibility of XANES experiments on diluted samples (trace metals) although more data would be necessary to precisely conclude on the nature of Fe ligands in yeast SOD-mutants and wild type cells.

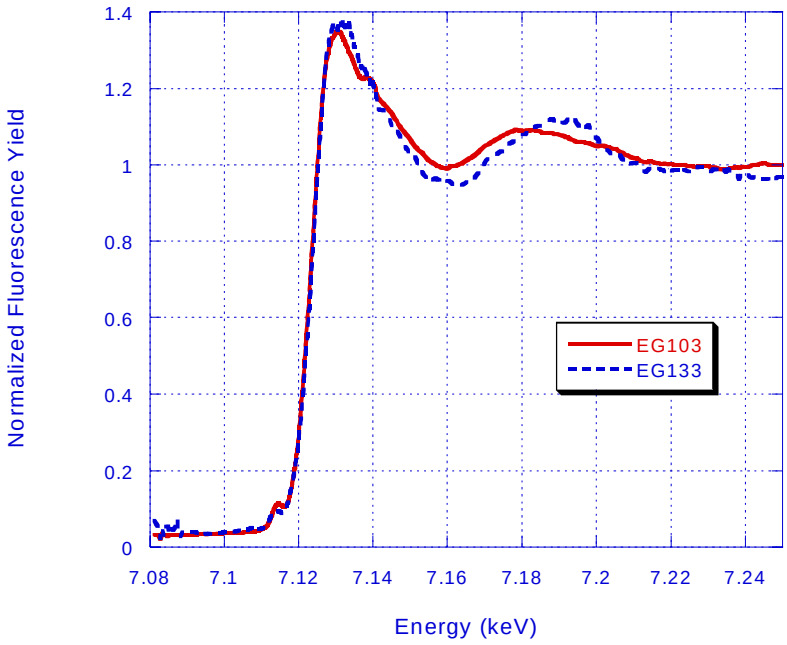

Figure 2. Normalized Fe K-edge XANES spectra from samples EG103 (wild type *S. cerevisiae*), and EG133 (double mutant Cu-Zn SOD/Mn SOD deleted).