Project Report

Project title: Microtomography of Pancreatic Islets By X-ray Phase-Contrast Imaging
Approved No.: MD891
Beamline: ID17
Maín Proposer: Enming ZHANG

Summary:

Islet function is damaged during the process of diabetic development and this is reflected on their morphology. To measure the size and number of islets in whole pancreas in the healthy and diabetic rodents, we have performed the X-ray phase contrast imaging on the samples at ID17 beamline station in ERSF. We clearly observed the islets in the pancreas with the imaging and primarily estimated that the number of islets were reduced at diabetic conditions. However, quality of the images was rather lower than expected and is chanllenge to make segmentation automatically by using software. Alternatively we are currently trying to use different manual-track methods to make the segmentation.

β Cell Mass in Diabetic Rodents: Experiment Protocol

Mice (C57bl):
Pancreas 4% - PFA

Mice were either injected with streptozotocin (STZ) for 1 day and sacrificed on the 7th day, or injected with STZ for 5 days and sacrificed the 6th. Control mice were not injected with anything. All mice were weighed and their blood glucose levels were measured before sacrifice.

Preparation:

- Sample is pre-soaked with ethanol after harvest.
- Sample is placed in 0.8 ml 4% PFA in a horizontally positioned 1.5 ml Eppendorf tube. Avoid bubbles.
- The lid is closed and the tube is positioned upside down on the stage ready for x-ray.

Rat:
Pancreas 4% - PFA

Young and old Wistar and GK rats were used. All rats were weighed and their blood glucose levels were measured before sacrifice.

Preparation:

- Sample pre-soaked with ethanol after harvest was placed at room temperature for 1 hour.
- Sample is then placed in 3-4 ml 4% PFA in a 5 ml vial. Excess PFA was removed.
- The lid is closed and the vial is positioned on the stage ready for x-ray.

β Cell Mass in Diabetic Rodents: Images Acquisition

After placing the sample tube/vial on a marked tape on the stage followed by safety procedures, images were taken under the following settings:

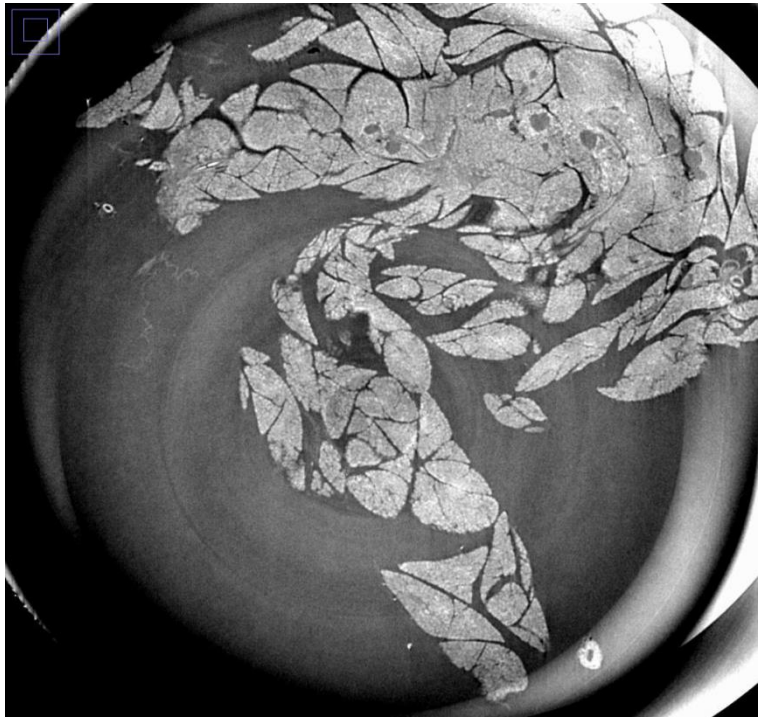
- Stage rotation: every 45°
- Rotation speed: 0.3°/sec
- Maximum rotation: 180°
- Image integration time: 0.1
- 2000 projections/stage, 5-10 stages, 3.2 mm frame height, 15.97 mm frame width
- Pixel size: 7.8 µm

First the start of the scanning point is determined which is usually from the base of the stage. After starting the x-ray, the machine automatically acquires images by rotating the stage at given speed and angle, and firing x-ray. The images are then combined and processed for analysis. All raw images acquired are in .edf format.

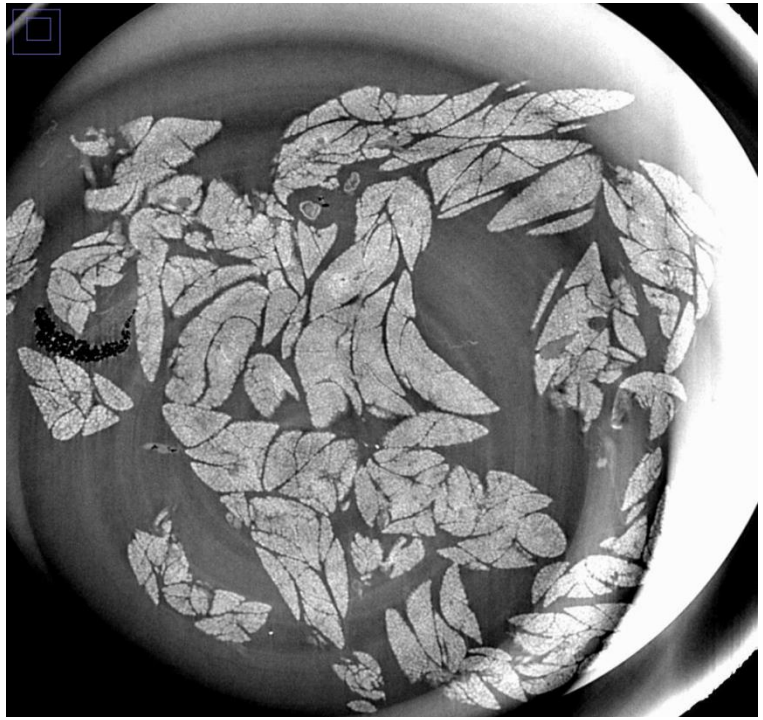
Sample number, Activity, Date, Time, Range (mm), Stage, and Current (mA) were recorded for each sample.

β Cell Mass in Diabetic Rodents: X-Ray Images

Control



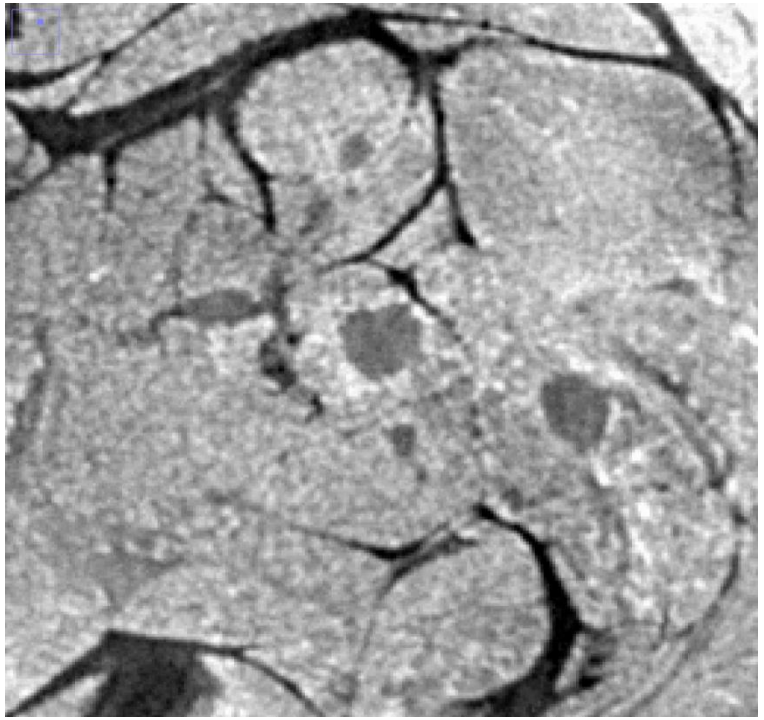
1 day STZ



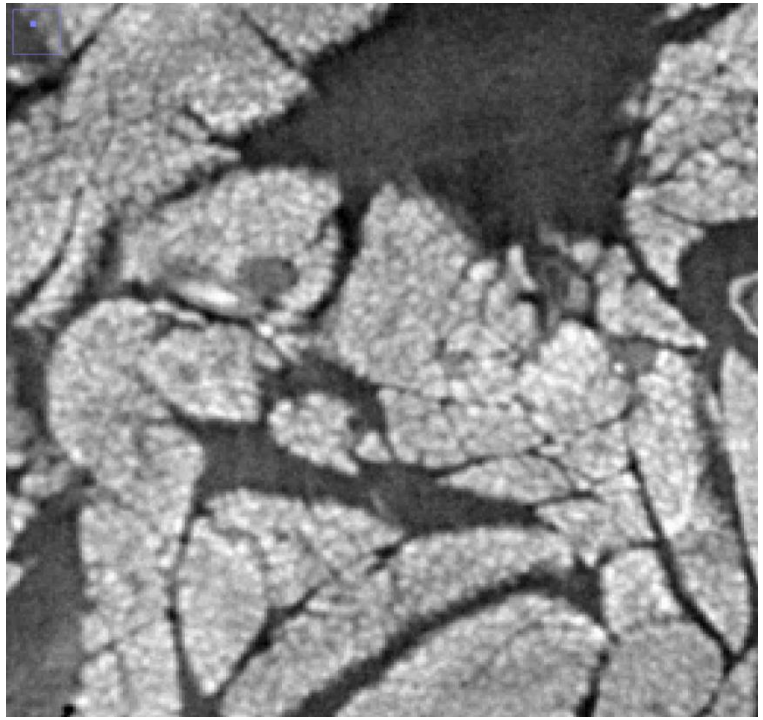
5 day STZ

β Cell Mass in Diabetic Rodents: X-Ray Images

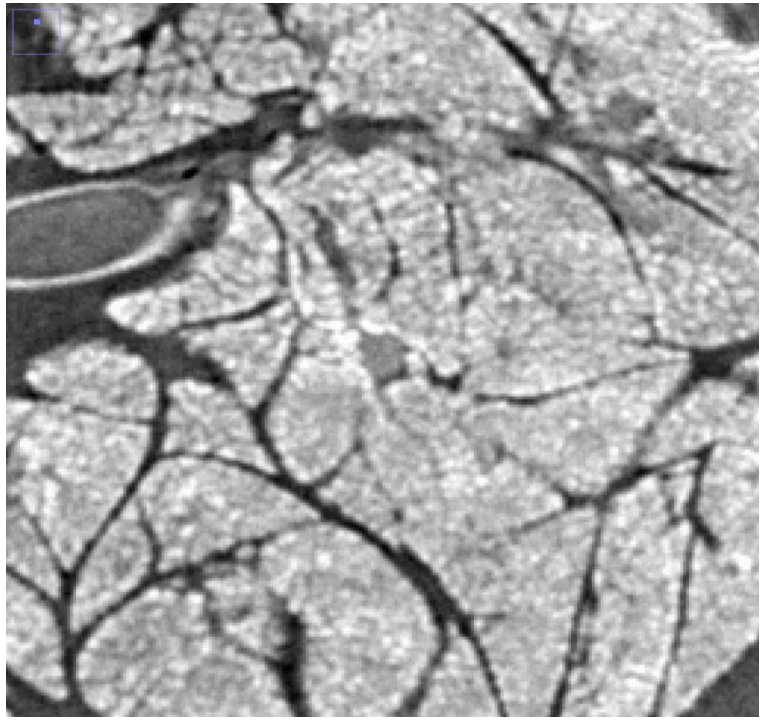
Control



1 day STZ



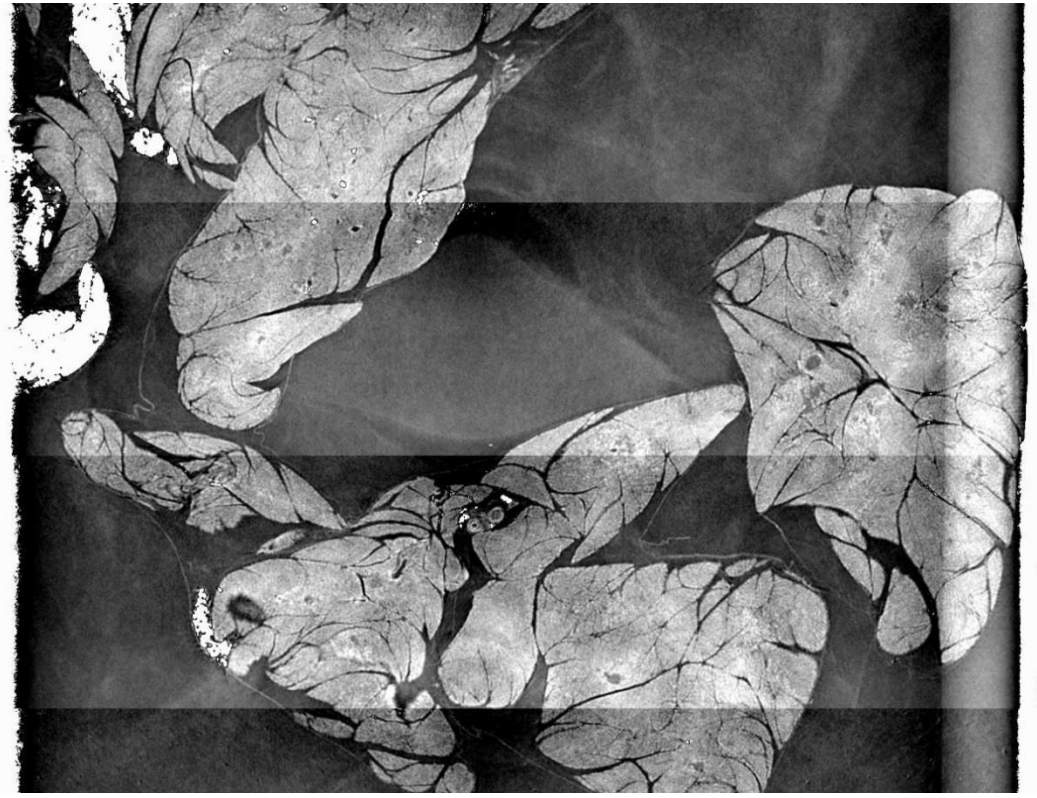
5 day STZ



β Cell Mass in Diabetic Rodents: X-Ray Images

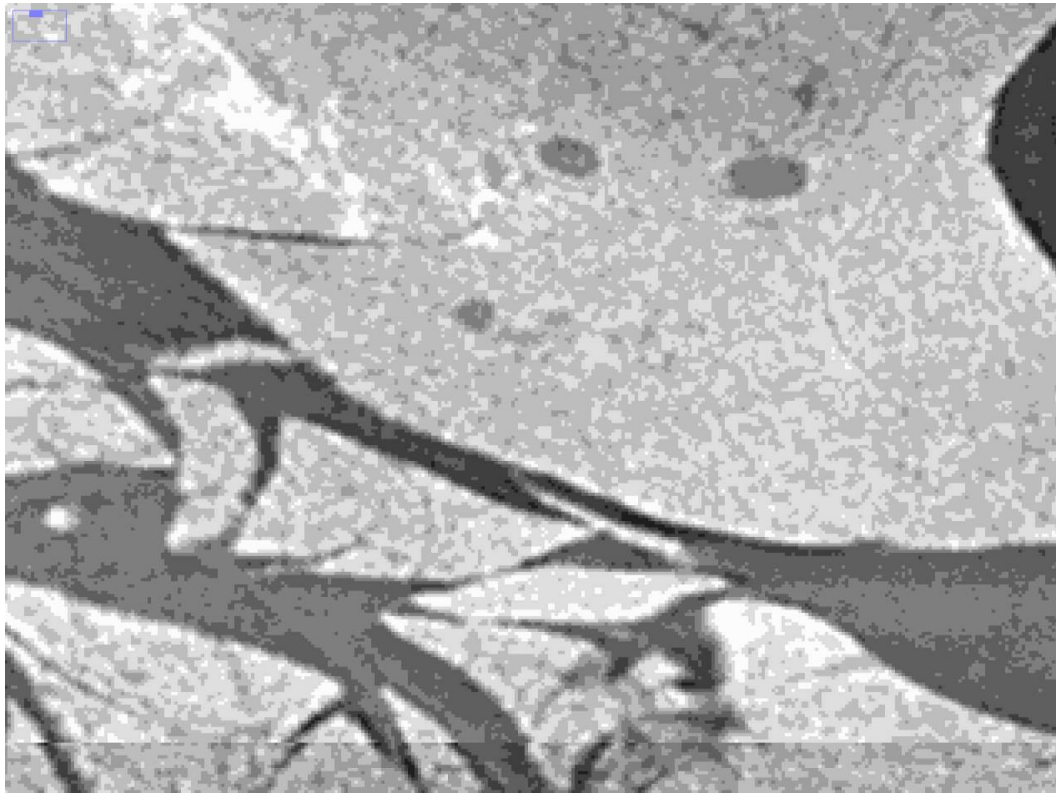
Wistar

GK

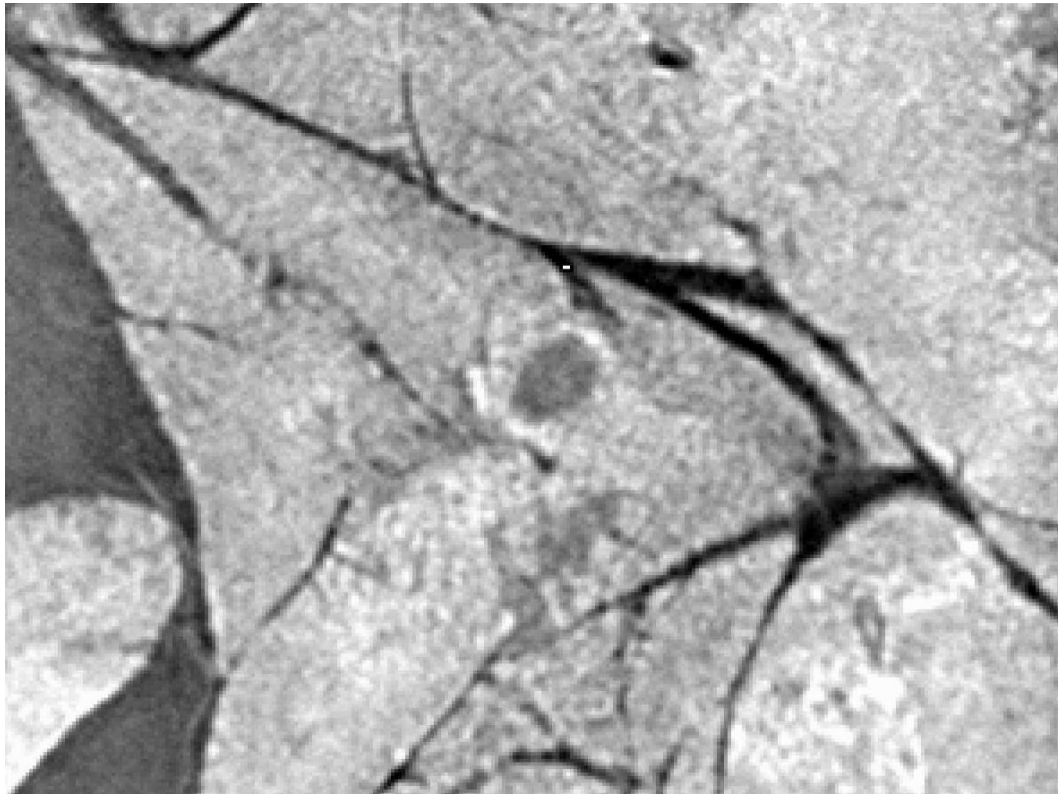


β Cell Mass in Diabetic Rodents: X-Ray Images

Wistar



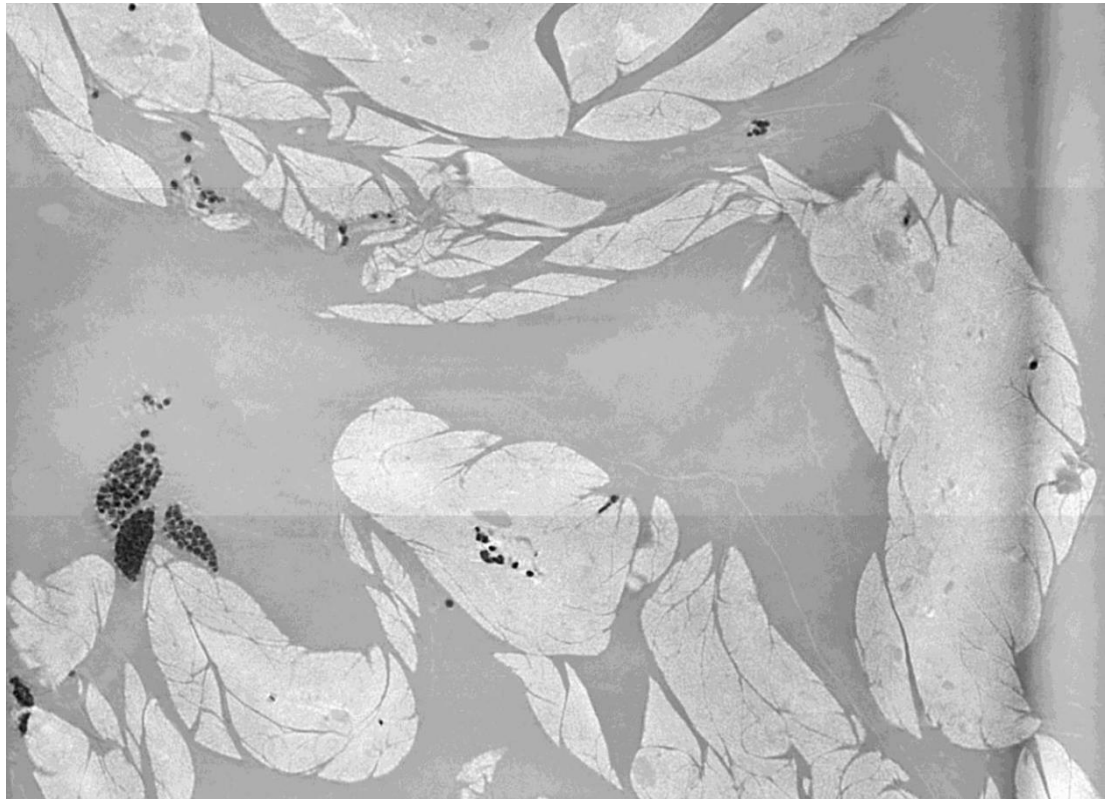
GK

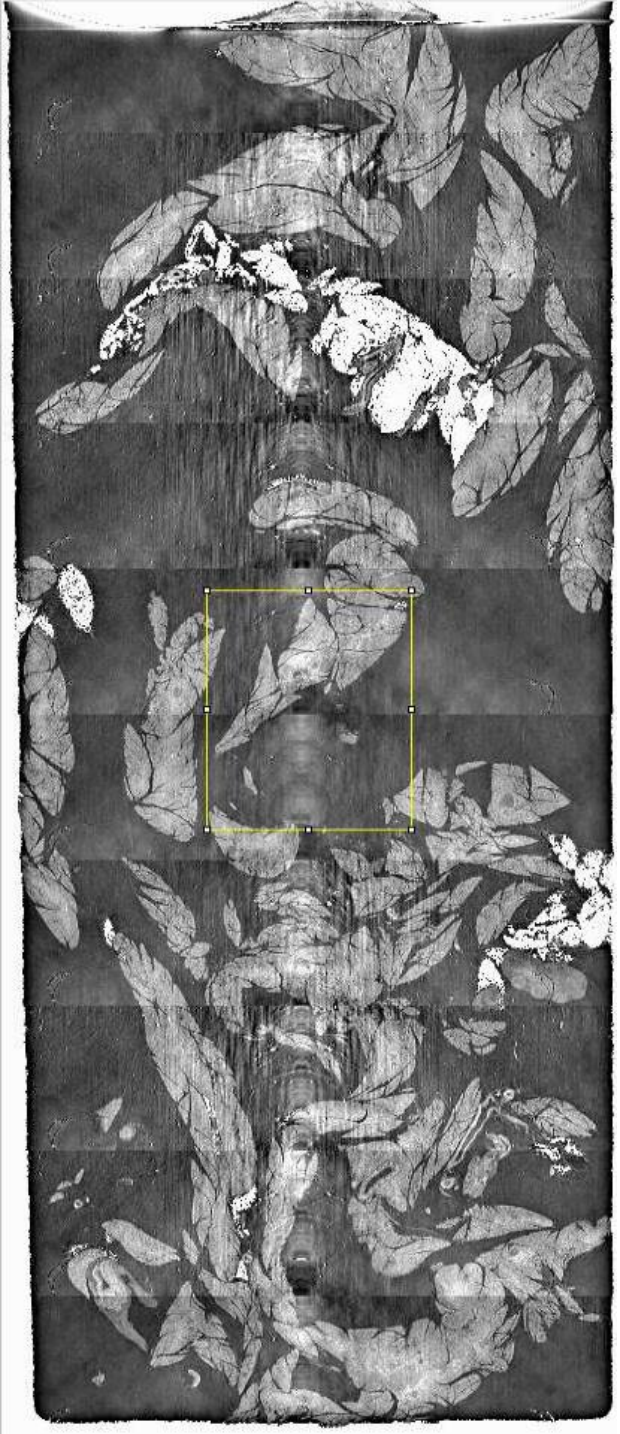


β Cell Mass in Diabetic Rodents: X-Ray Images

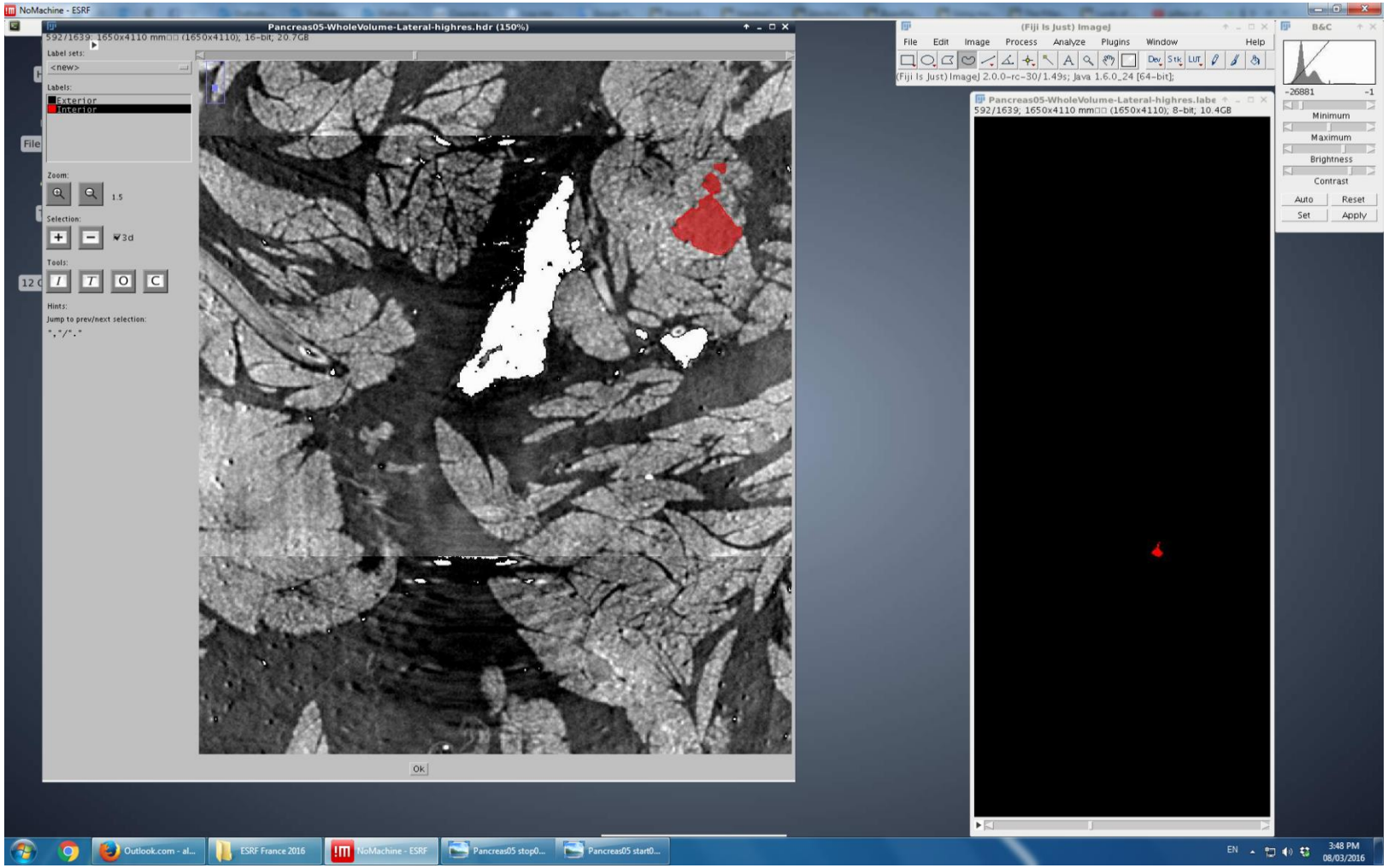
High contrast

Low contrast





β Cell Mass in Diabetic Rodents: Segmentation manually



β Cell Mass in Diabetic Rodents: Analysis Challenges

Since all islet selections were made manually, one area (yellow rectangle shown on the previous slide) of a sample takes approximately 3 weeks. In order to select the islets faster, a software capable of accurately recognizing and selecting islets is required.

The size (volume) and number of islets selected will have to be calculated using 3D modeling.