Neutron Radiography and X-ray Analysis of Siliceous Marine Sponges: *Dragmacidon lunecharta*

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Overview

- Climate change and human activity are causing rapid changes to the world's oceans and the ecosystems contained within them.
  - Pollutants such as cadmium are of particular concern.
- Demospongiae, the largest class of Porifera (Sea Sponges), are found in oceanic waters from the tropics to Antarctica.

Figure 1. Live *D. lunaecharta*

Objectives

- Find new techniques to evaluate sponges as an effective biomarker for Cd pollution.
- Determine if NAA can be used to quantify Cd uptake levels.
- Determine the ability of neutron radiography to detect suspected Cd uptake in a biological system.
  - Is there a preferential region of disposition within the sponge?
- Image the Cd uptake pathways without destroying the specimen.

Collaboration with Nuclear Facilities

- Idaho State University (ISU) - AGN-201- 5W
  - Initial NAA studies
- UMass-Lowell Research Reactor (UMLRR) -1MW
  - NAA
- Nuclear Radiography Reactor (NRAD) at the Idaho National Lab (INL) – 250kW TRIGA
  - Radial Beam Digital Radiography
  - Transfer Method (Indirect) Neutron Radiography (Film)
- McClellan Nuclear Research Center (MNRC) - 2MW TRIGA
  - Direct Neutron Radiography (Film)
- FRM-II Technical University- Munich - 20 MW
  - Cold Neutron Computed Tomography (nCT)

Background

Why Sponges?

- Multiple studies, based on destructive chemical analysis, have shown sponges to be useful as bio-indicators for heavy metal accumulation due to their ecological role as biofilters.
  - Internal structure allows them to filter water even if their choanocytes (cells responsible for management of water flow) are incapacitated.

Background: Internal Structure

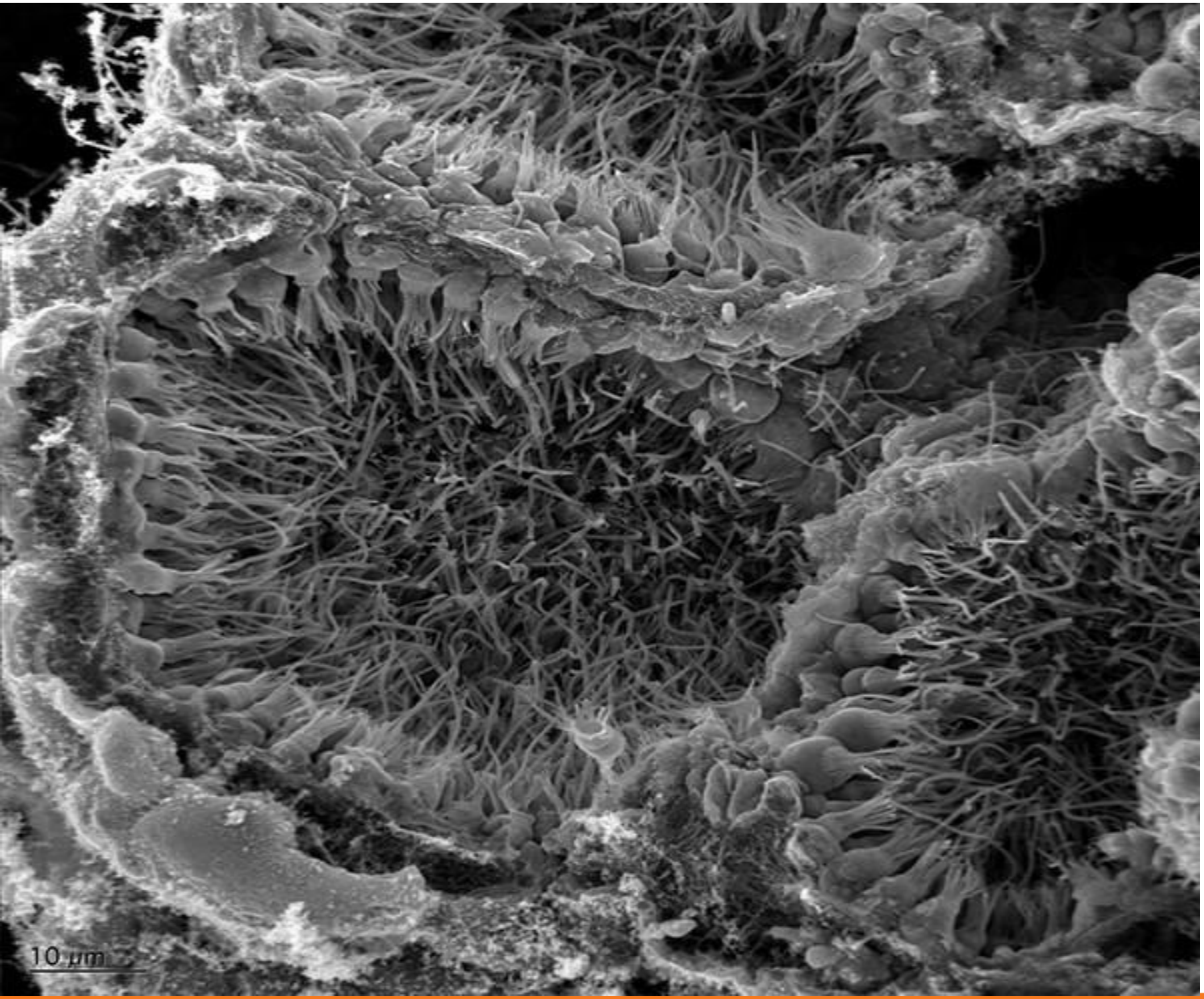


Figure 2. Image taken in situ using a scanning Electron microscope with a resolution of 10 µm. In this image the choanocytes are the cells that appear to have a collar and and a tail. This tail is whipped to create the unidirectional water flow used by the sponge to obtain oxygen and necessary nutrients. Dayel (2011)

Background: Internal Structure

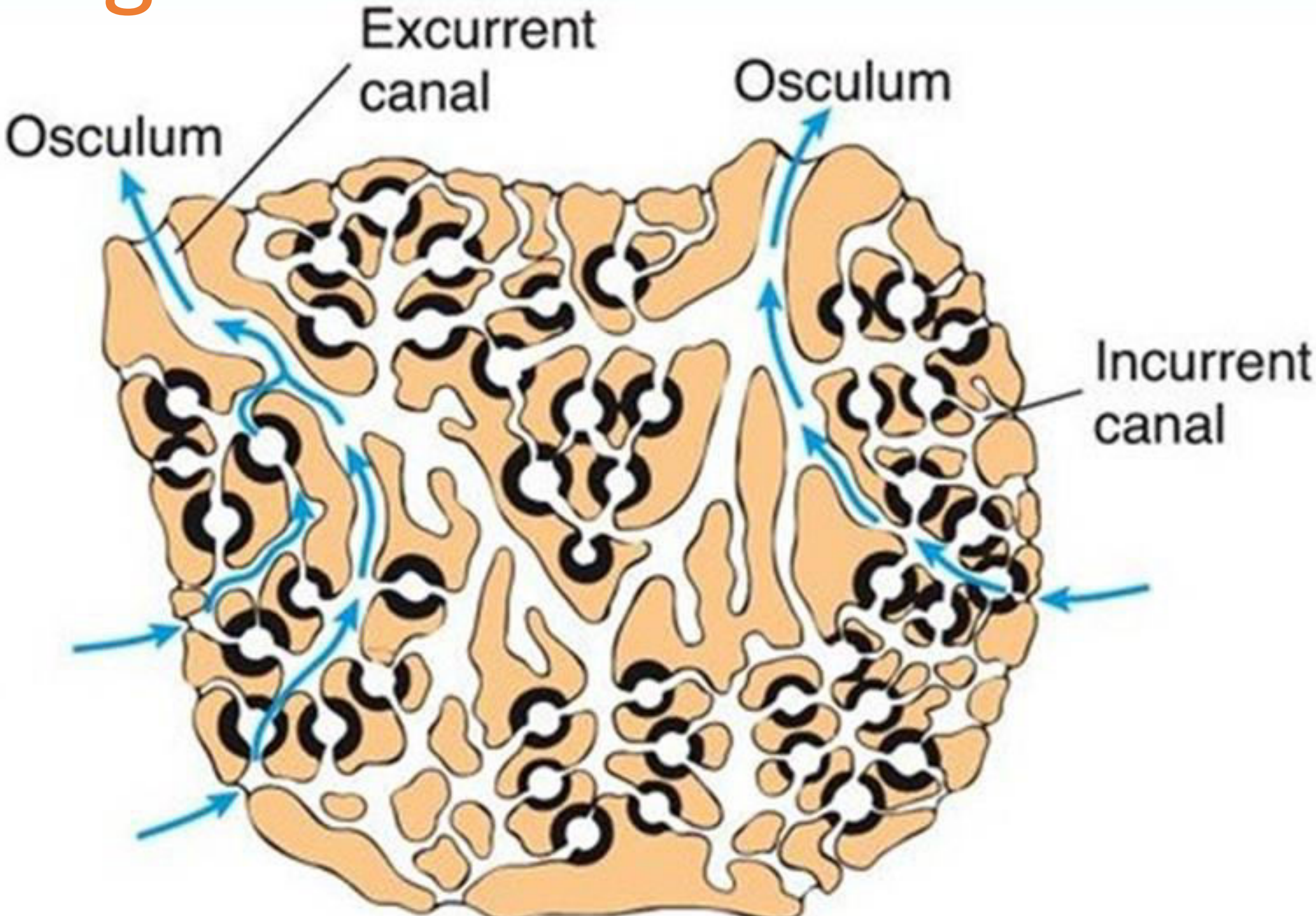


Figure 3. Leuconoid structure of a demosponge. Blue arrows denote water flow in an alive and healthy sponge. Death or damage of choanocytes can lead to multi-directional waterflow. Without functioning choanocytes, other cells may continue to function in a limited capacity until total cellular death occurs. Barnes (1982)

Background: Previous Research

Why NAA?

- Silicon based sponges are notoriously difficult to accurately analyze using traditional chemical analysis.

X-ray vs. Neutron Radiography

- Both are nondestructive.
- Each radiographic technique exposes different characteristics of the same object.
- X-ray images useful for identifying structures of interest to be the focus of neutron imaging.

Background: Previous Research

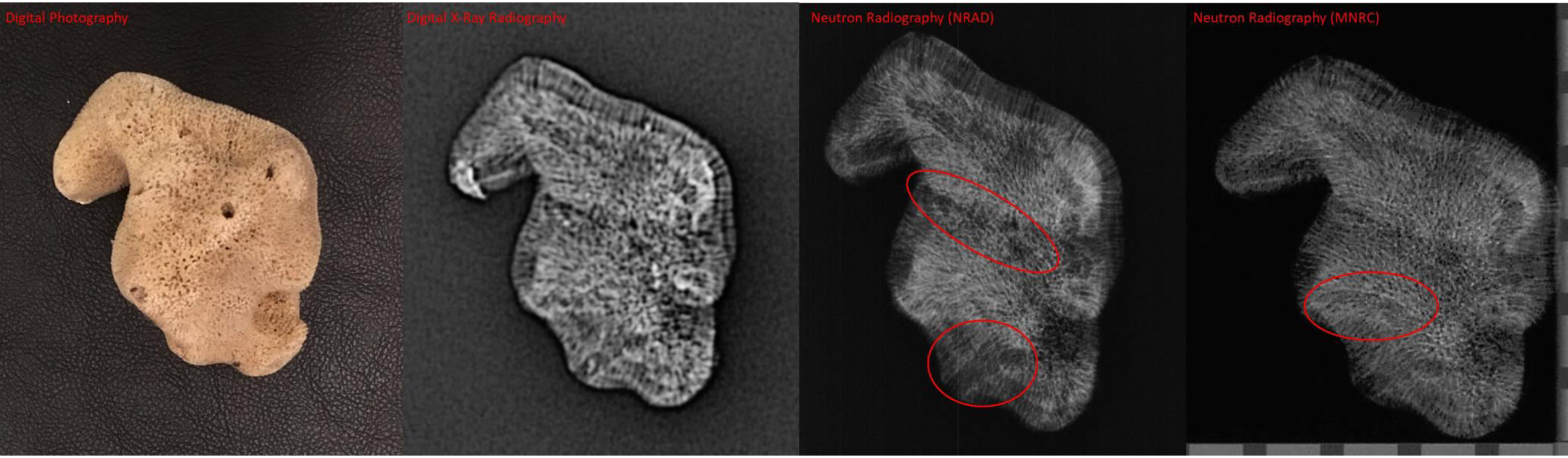


Figure 4. (Left to Right) Digital photography shows a dried *D. lunaecharta* as viewed by the naked eye. The interactions with the electrons of the atoms of which the specimen is comprised allow the digital x-ray radiography to reveal surface features and some internal structures of interest. Neutron radiography provides increased detail of these structures through the neutron interactions with the nuclei of the same atoms. Slightly different orientations during imaging and different L/D ratios provided additional views of the sample when neutron radiography was performed at both the Neutron Radiography (NRAD) reactor and the reactor at the McClellan Nuclear Research Center.

Background: Choice of Toxin

Why Cadmium?

- Cd is #7 on The Agency for Toxic Substances and Disease Registry top 20 list and is a group 1 human carcinogenic.
- Multiple sources of pollution:
  - released as by product of burning coal and Pb and Zn mining
  - common component of commercial fertilizers, insecticides, and fungicides
  - released from natural geologic formations as ocean pH decreases, driving the dissolution of bedrock
- Cd has a high neutron absorption cross section compared to other elements contained within biological systems allowing it to be more visible on images generated via neutron radiography.

Background: Choice of Toxin

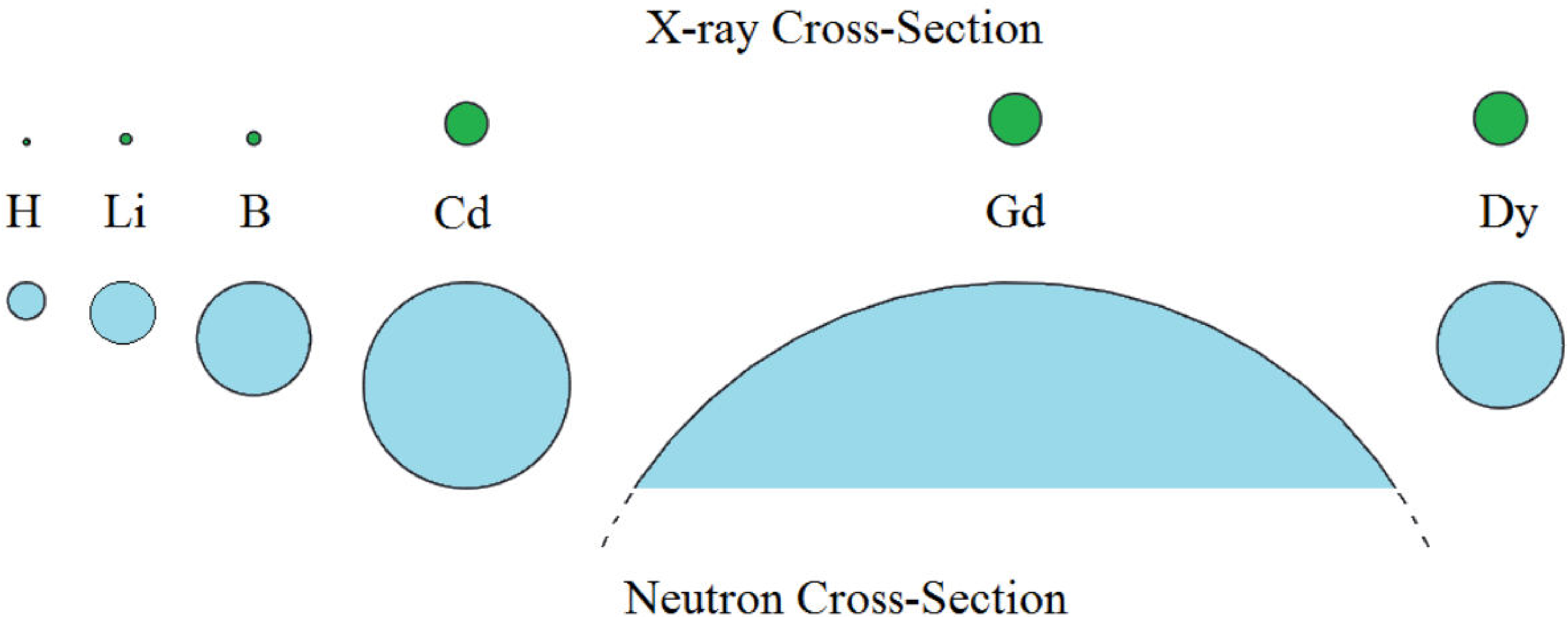


Figure 5. Illustration of x-ray attenuation verses neutron absorption cross section for six elements. Craft (2018)

Background: Sample Preparation for NAA

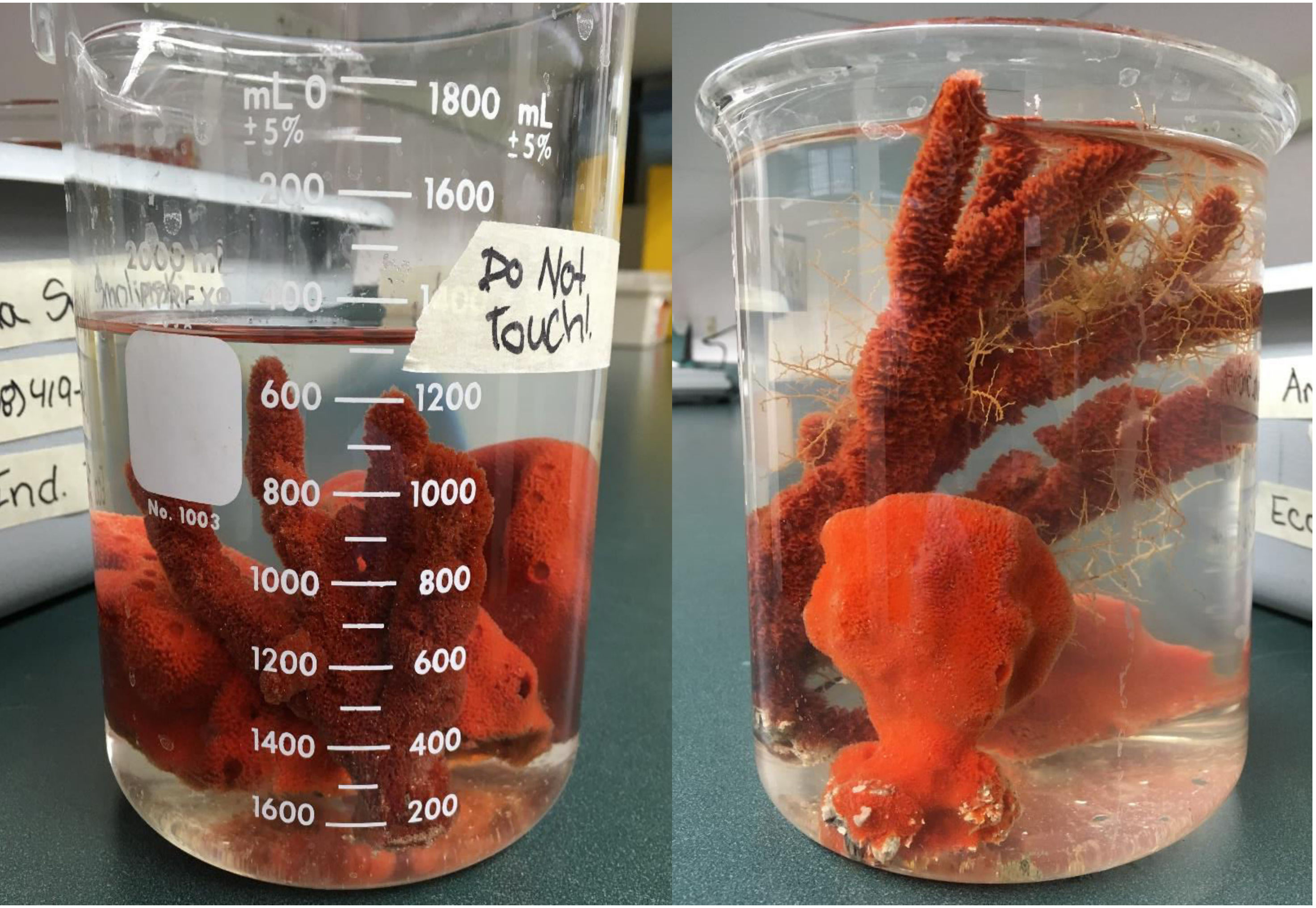


Figure 6. (Left to Right) To test the hypothesis that the sponges would absorb Cd in appreciable amounts, two red ball sponges and one red tree sponge were exposed to 1000 ppm Cd salt water mix. The control consisted of two red ball sponges and one red tree sponge. The sponges were soaked for five days, at which time the controls began to show signs of external damage.

Background: Results of NAA

Table 1. As hypothesized, the exposed sponges did absorb appreciable amounts of Cd. This data is a portion of the NAA performed by Dr. Nelson G. Eby at UMass-Lowell. Samples with nd (not detectable) are the control samples.

Sample	Cd (ppm)
RBS-A	nd
RBS-B	nd
RBS-C	692
RBS-D	838
RTS-A	nd
RTS-B	1120

Methods: Sample Preparation

Table 2. $CdCl_2$ concentration (ppm) by well. Specimen numbers correspond to well number.

Well	$CdCl_2$ concentration	Well	$CdCl_2$ concentration
1	1000	4	2000
2	0	5	3000
3	1500	6	4000

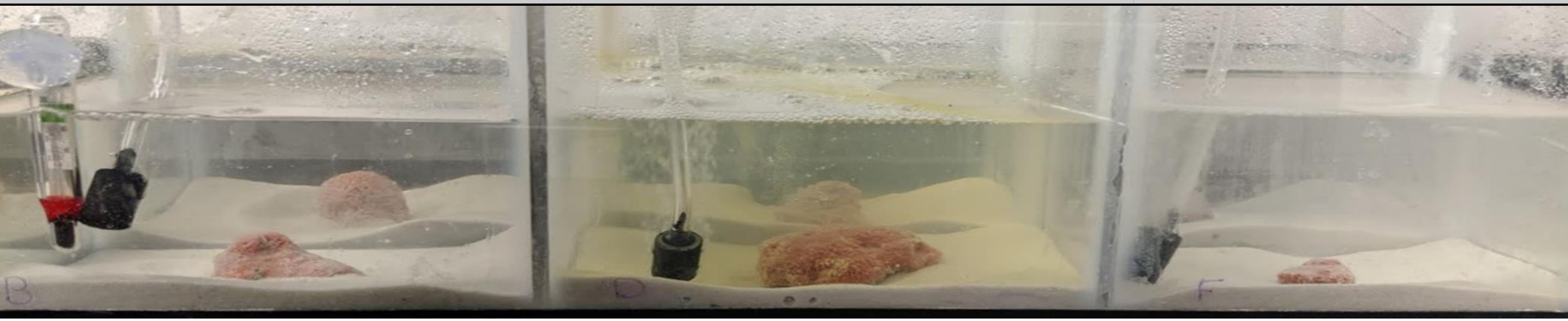


Figure 7. Porifera Isolation Tank (PIT) used individual wells to separate the sponges while attempting to create a less artificial environment to offset the stress induced by larger concentrations of Cd. The randomized concentrations were intended to test absorption thresholds. Every attempt was made to equalize non–Cd parameters. Stress due to the shipping process led to external cellular damage on several of the specimens. This damage may be responsible for the formation of a CdS precipitate that occurred in wells 3 and 4 (middle wells in above image).

Methods: Sample Preparation

Figure 8. After six days sponges were removed from the PIT and dried on a metal drying rack in a fume hood. The CdS precipitate is visible on specimens 3, 4 and, unexpectedly 5. Internal structures, as viewed through osculum, were determined to be intact.

Methods: Imaging

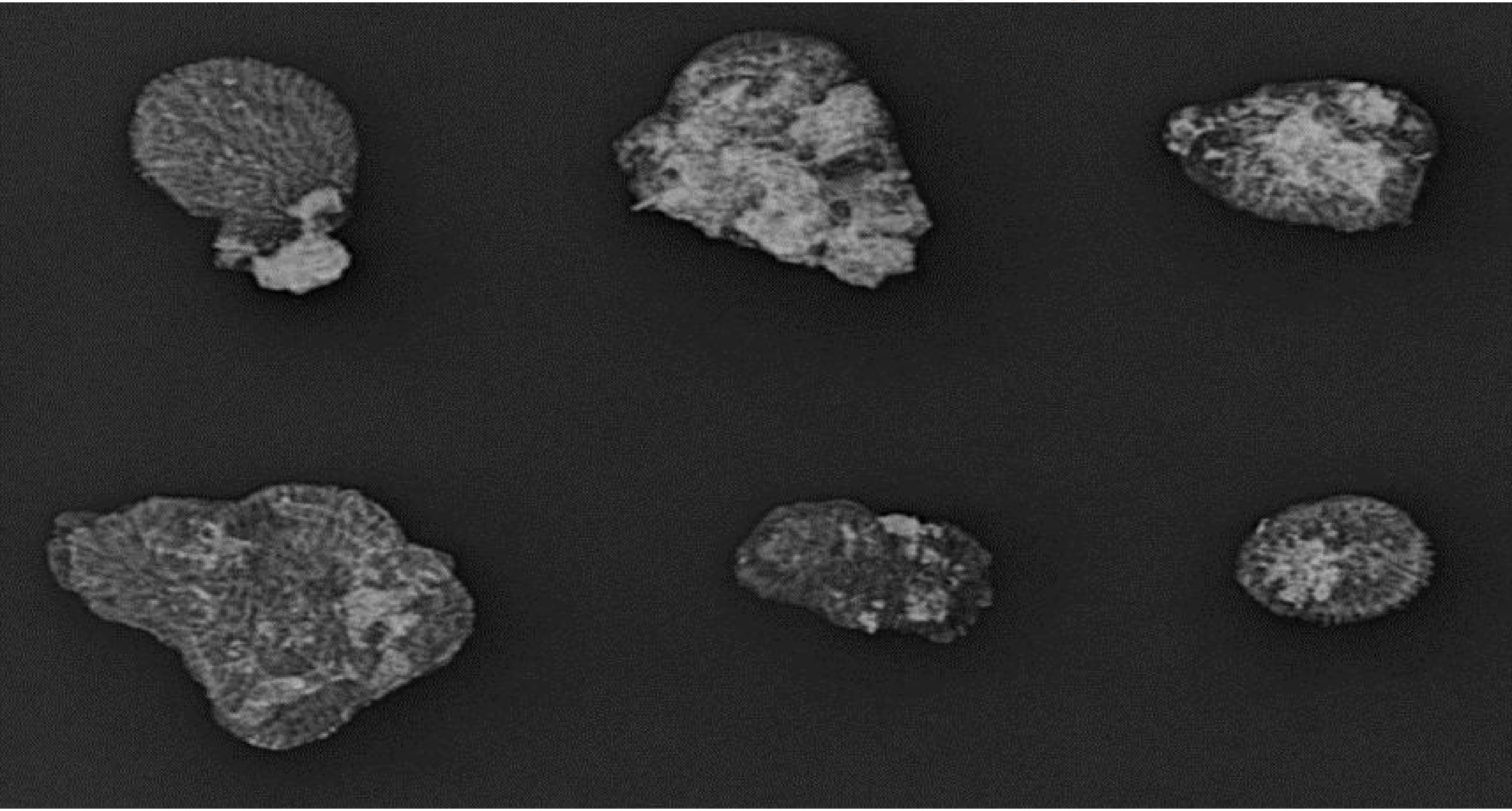


Figure 9. Digital x-ray image taken at 52keV. Multiple images were taken between 50 keV and 92 keV with no discernable increase in either internal or surface detail.

Methods: Imaging

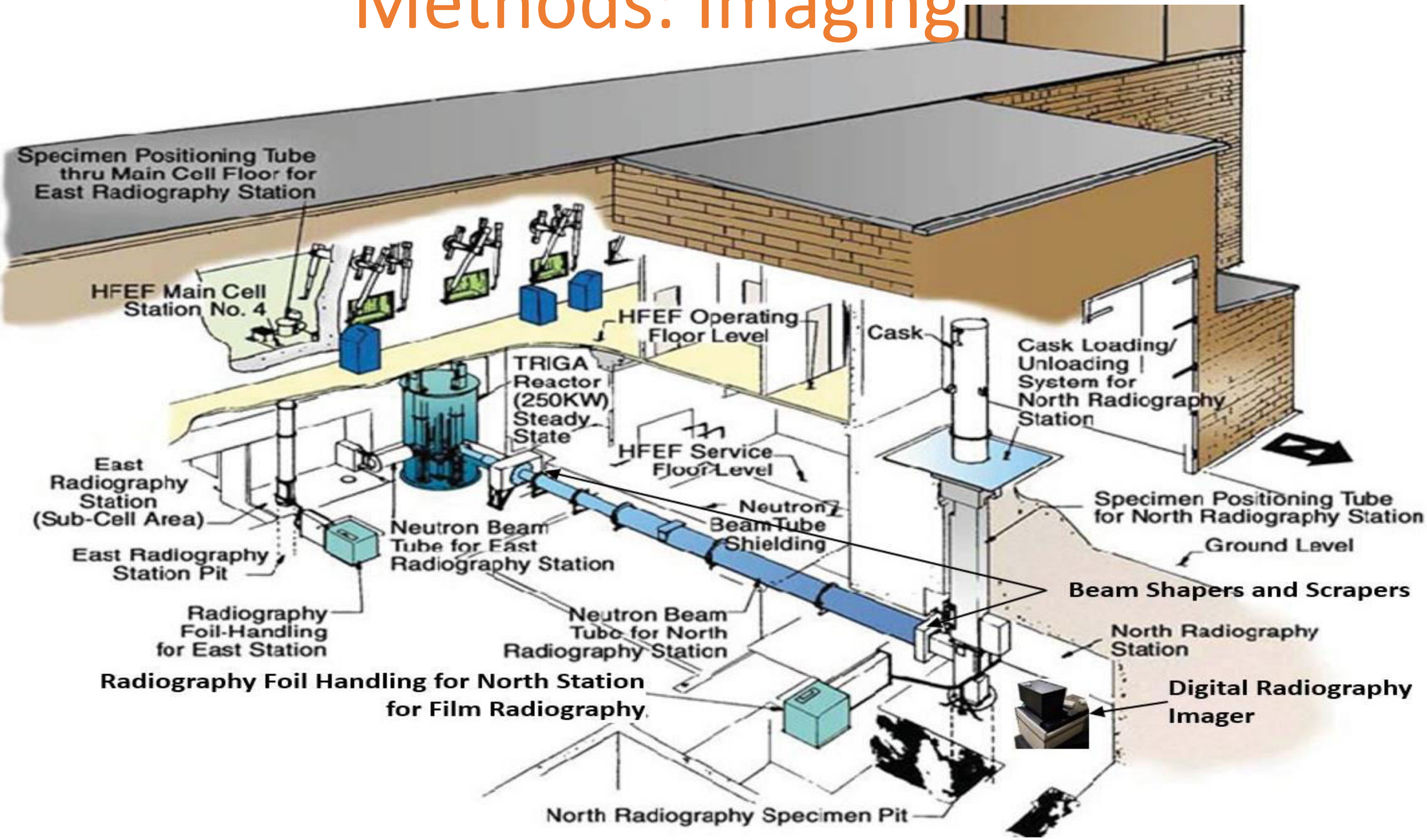
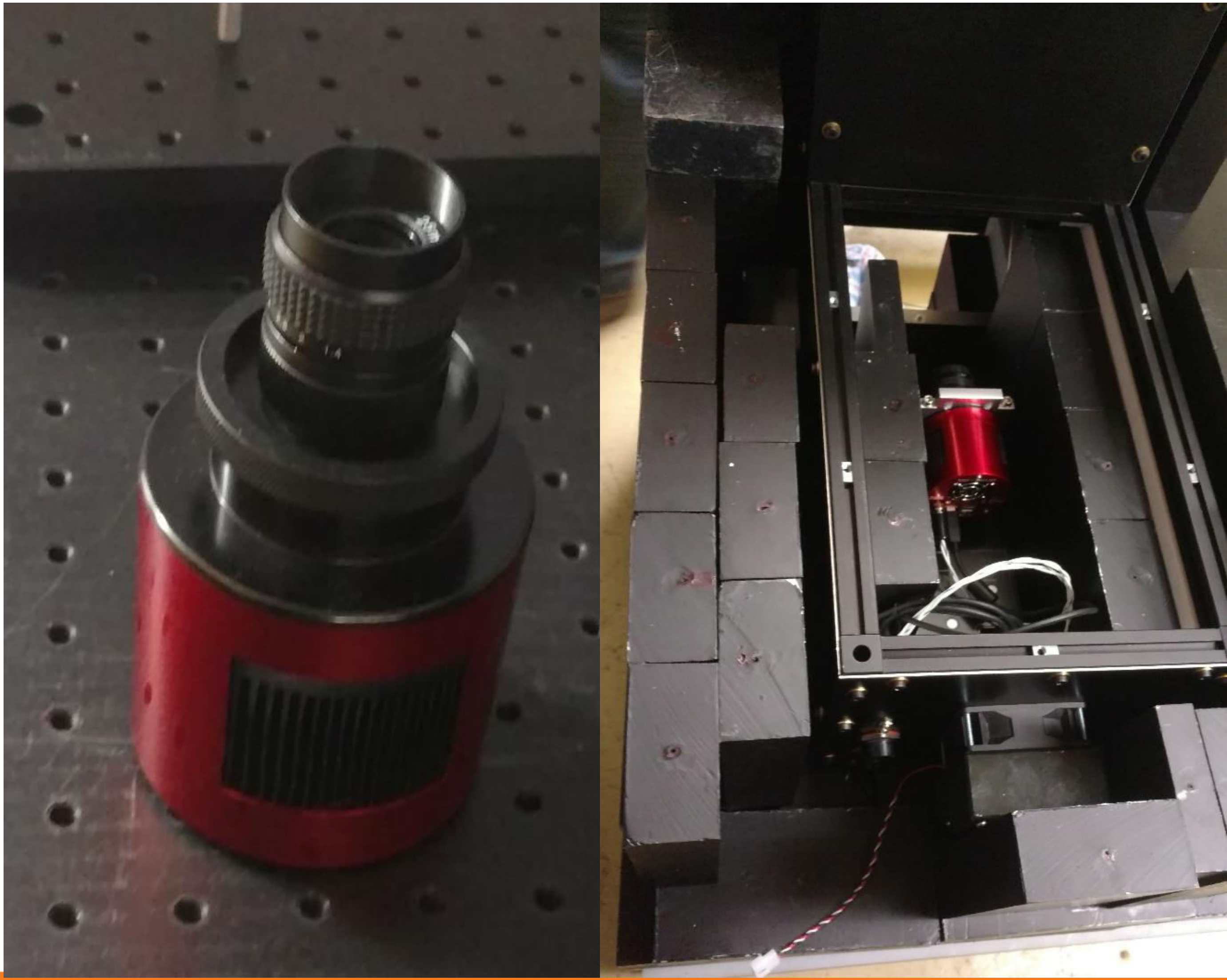


Figure 10. Neutron Radiography (NRAD) reactor facility layout. This facility is located at the Materials and Fuels Complex (MFC) in the INL. Currently there are two beam lines. Digital radiography was completed in the North Radiography Station (NRS) while indirect film radiography was completed in the East Radiography Station (ERS).

Methods: Imaging

Figure 11. (Left) ZWO ASI178MM cooled monochrome astronomy camera with 14-bit ADC, and extremely low noise. The pixel array is 3096 x 2080 with a 2.4 µm square pixel size. (Right) Camera is placed inside a shielded black powder-coated aluminum darkbox for radiation protection and light shielding during imaging. Image quality is dependent on a light-tight environment inside the darkbox.



Methods: Imaging

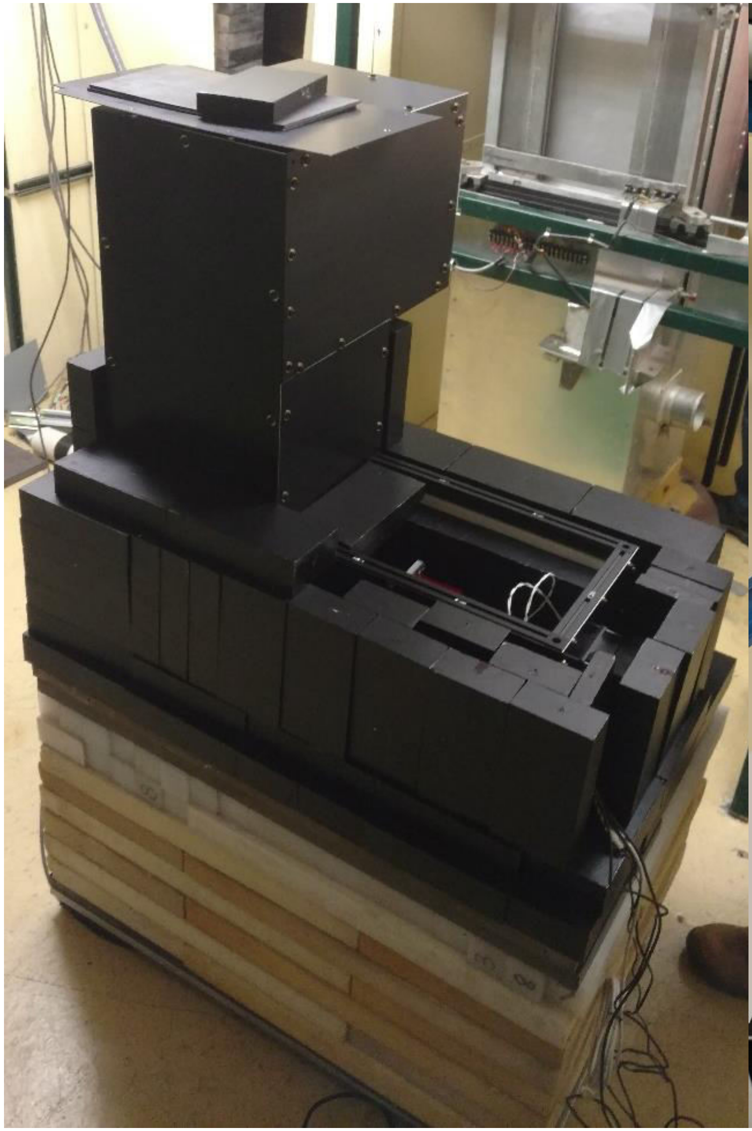


Figure 12. (Left) Double-periscope darkbox, in NRAD North Radiography Station (NRS), surrounded by powder coated lead bricks.
(Right) Double-periscope darkbox with the camera and mirror compartments open.

Methods: Imaging

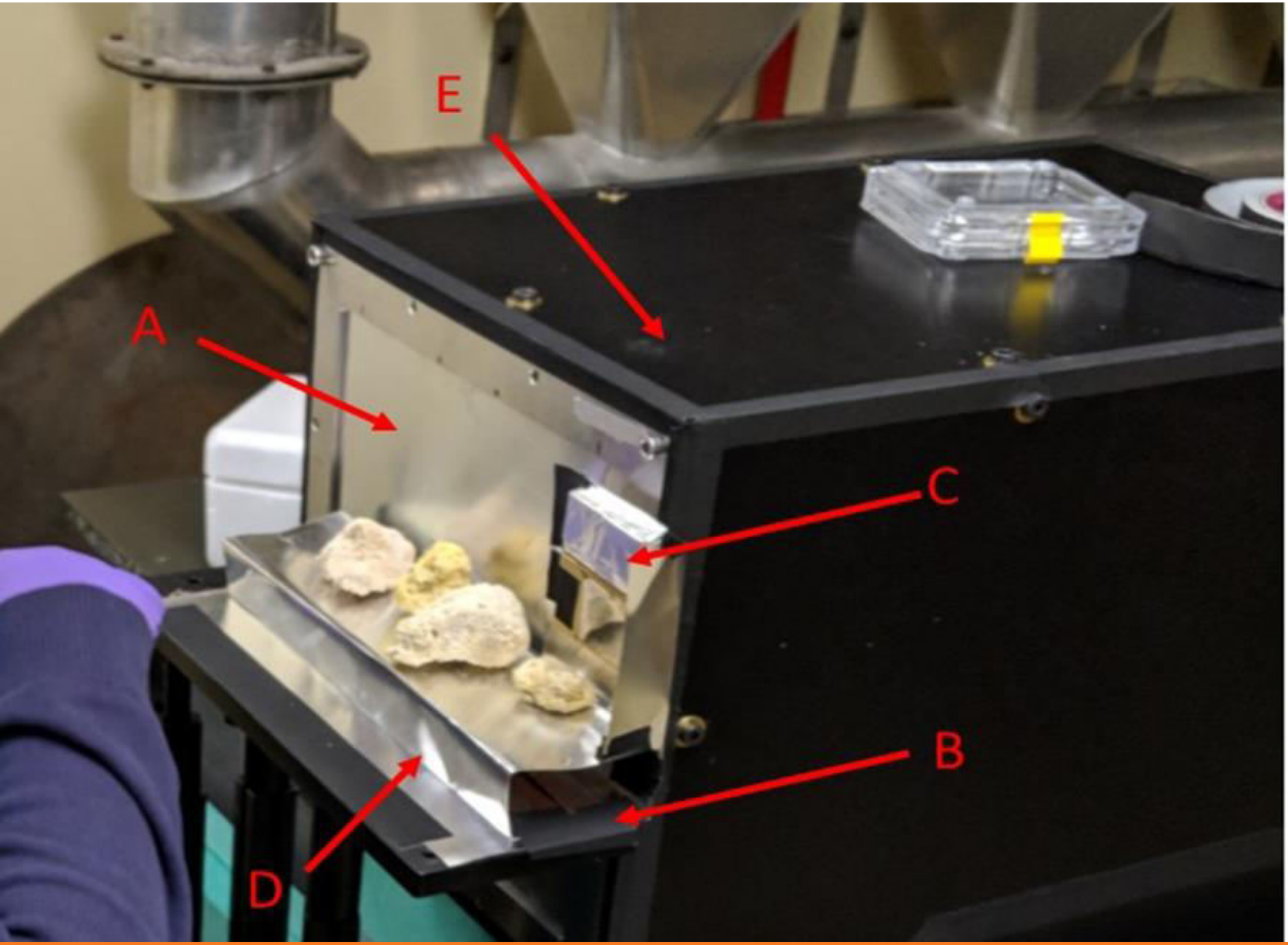


Figure 13.
(A) LiF-ZnS scintillator plate
(B) Linear stage
(C) Image Quality Indicators (IQIs)
(D) Aluminum sheeting
(E) Darkbox

Results: Imaging

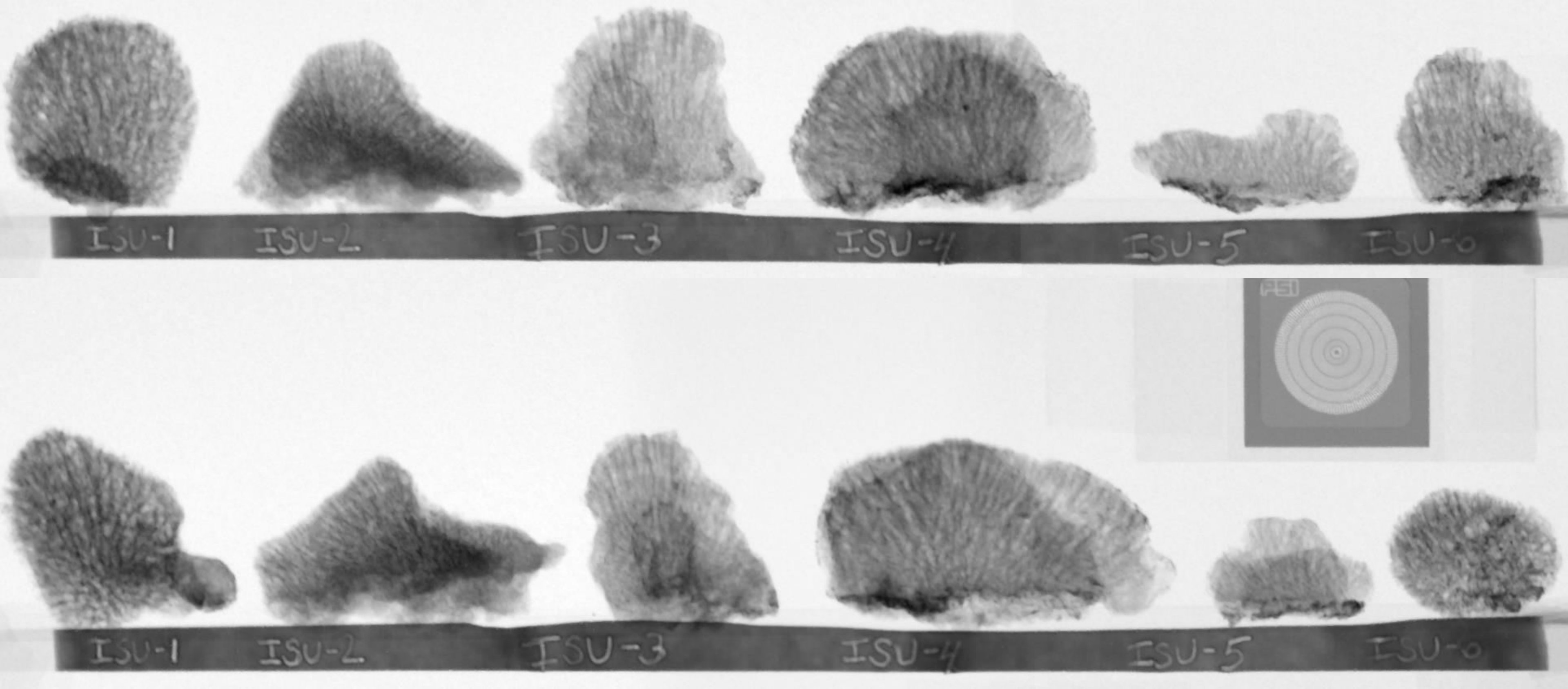
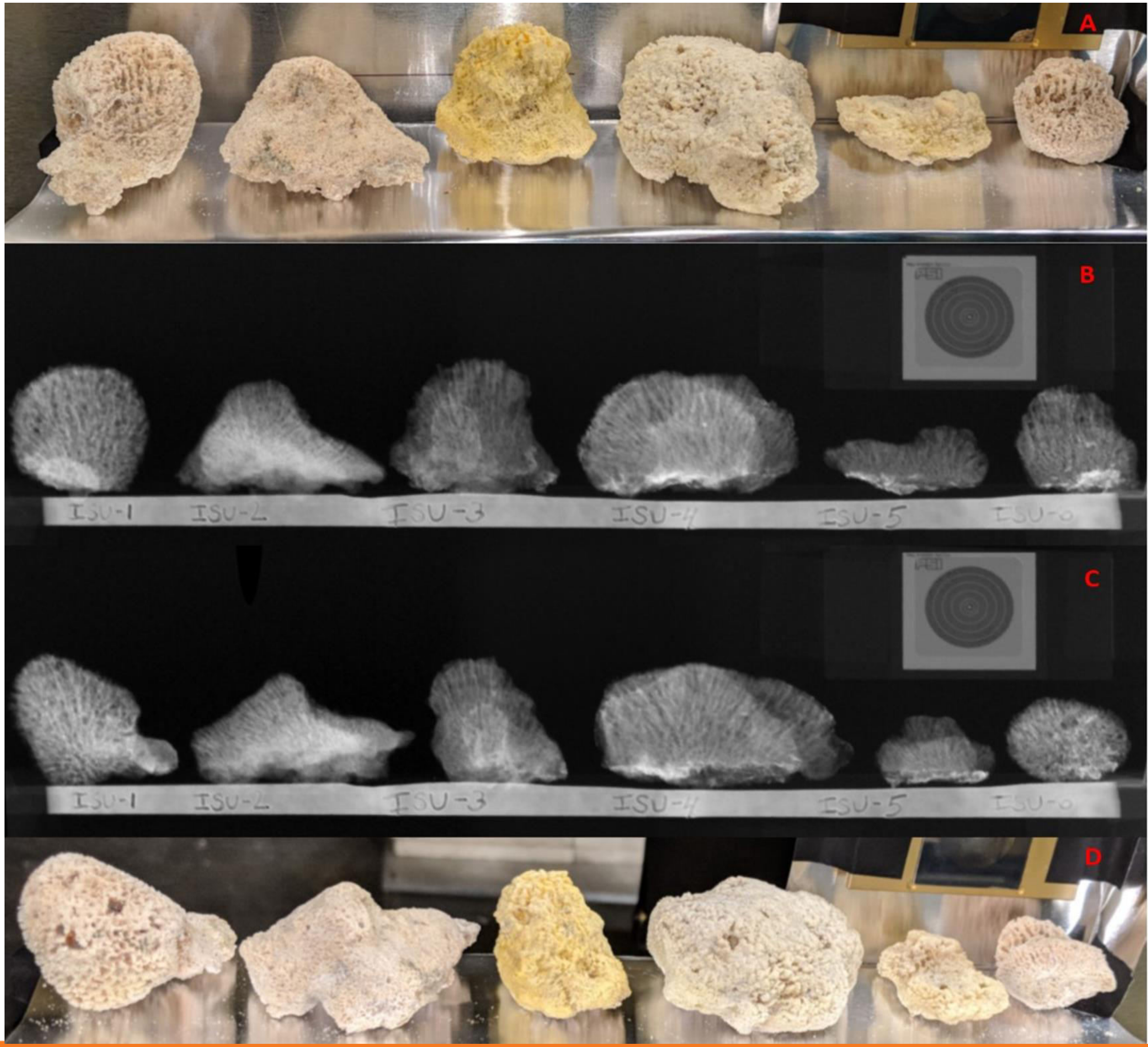


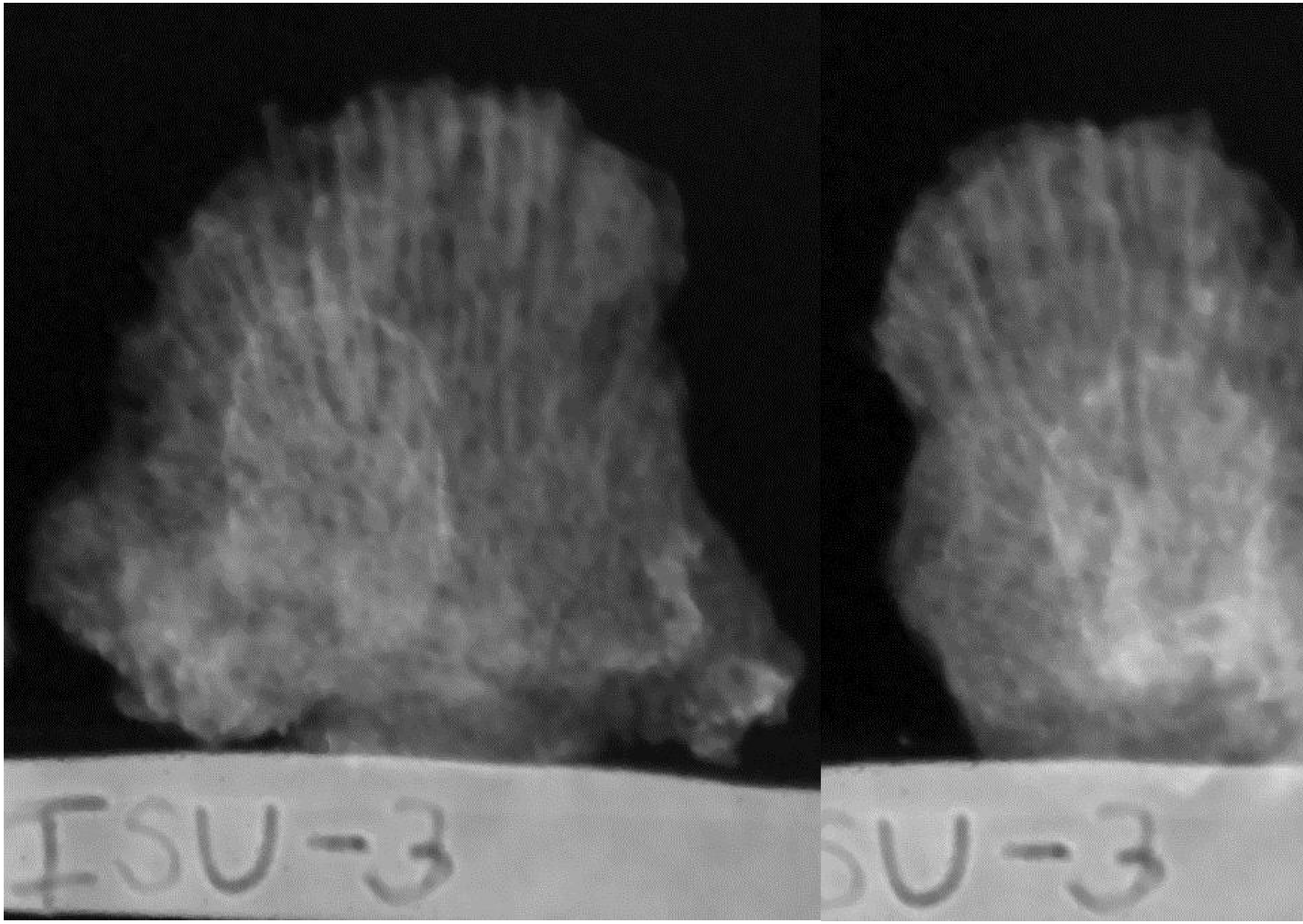
Figure 14. First and second digital neutron radiography images after post-processing. Sponges were rotated 90º counter-clockwise. The shape of specimen 6 necessitated laying it down after rotation. The IQI visible in bottom image indicates a resolution of 200-250 µm. Images taken with an L/D ratio of 185 and an exposure time of 300s.

Results and Discussion

Figure 15. (Top to bottom.)
(A) Digital image of specimen orientation for first digital neutron radiography image.
(B) First digital neutron radiography image after post-processing and inversion.
(C) Second digital neutron radiography image after post-processing and inversion.
(D) Digital image of specimen orientation for second digital neutron radiography image.



Results and Discussion



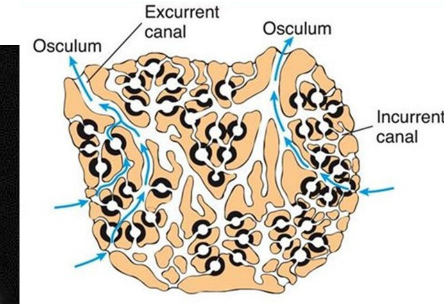


Figure 16. Side by side comparison of specimen 3 in the original imaging orientation (left) and after being flipped counterclockwise by 90º (right). The artifact in the center of each view is a suspected Cd deposit. Figure 3. leuconoid structure provided to illustrate the potential deposit geometry within the sponge.

Follow Up Radiography

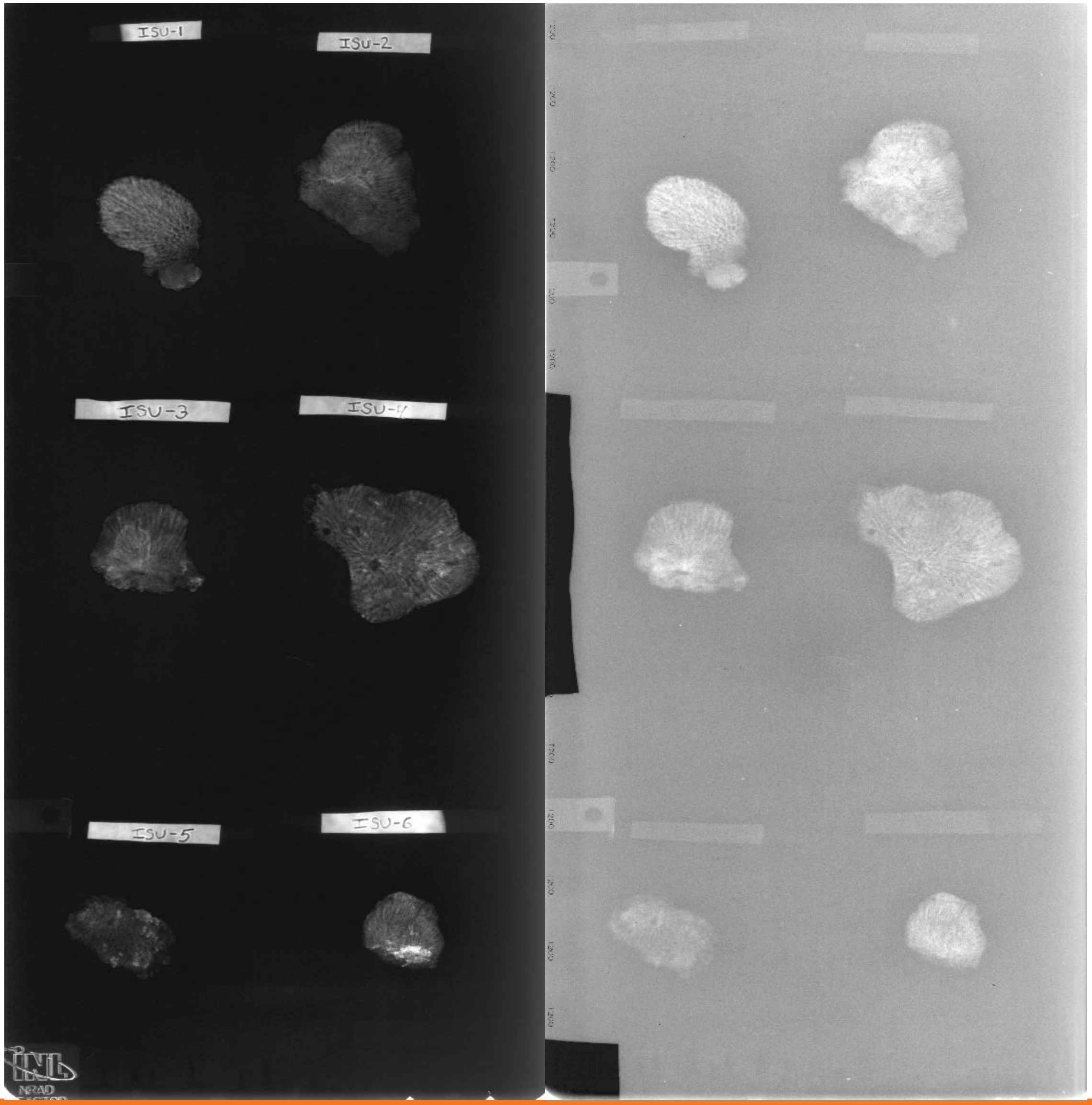


Figure 17. Processed film images of transfer method neutron radiography completed in the ERS at NRAD. The L/D ratio was 125 with a 22 minute exposure. Due to the size of the cassette used for this method, the orientation of specimens was slightly different than the digital radiography runs. The foils were allowed to decay overnight to achieve at least 5 half-lives of exposure from the foil to the film. Films were digitized at 16 bit with 1200 lines/inch.

(Left) Results of the dysprosium transfer foil used to transfer the image created by absorption of thermal neutron energies.

(Right) Results of the indium transfer foil, encapsulated by two sheets of Cd, to ensure transfer of only the image produced by epithermal neutrons.

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Follow Up Radiography

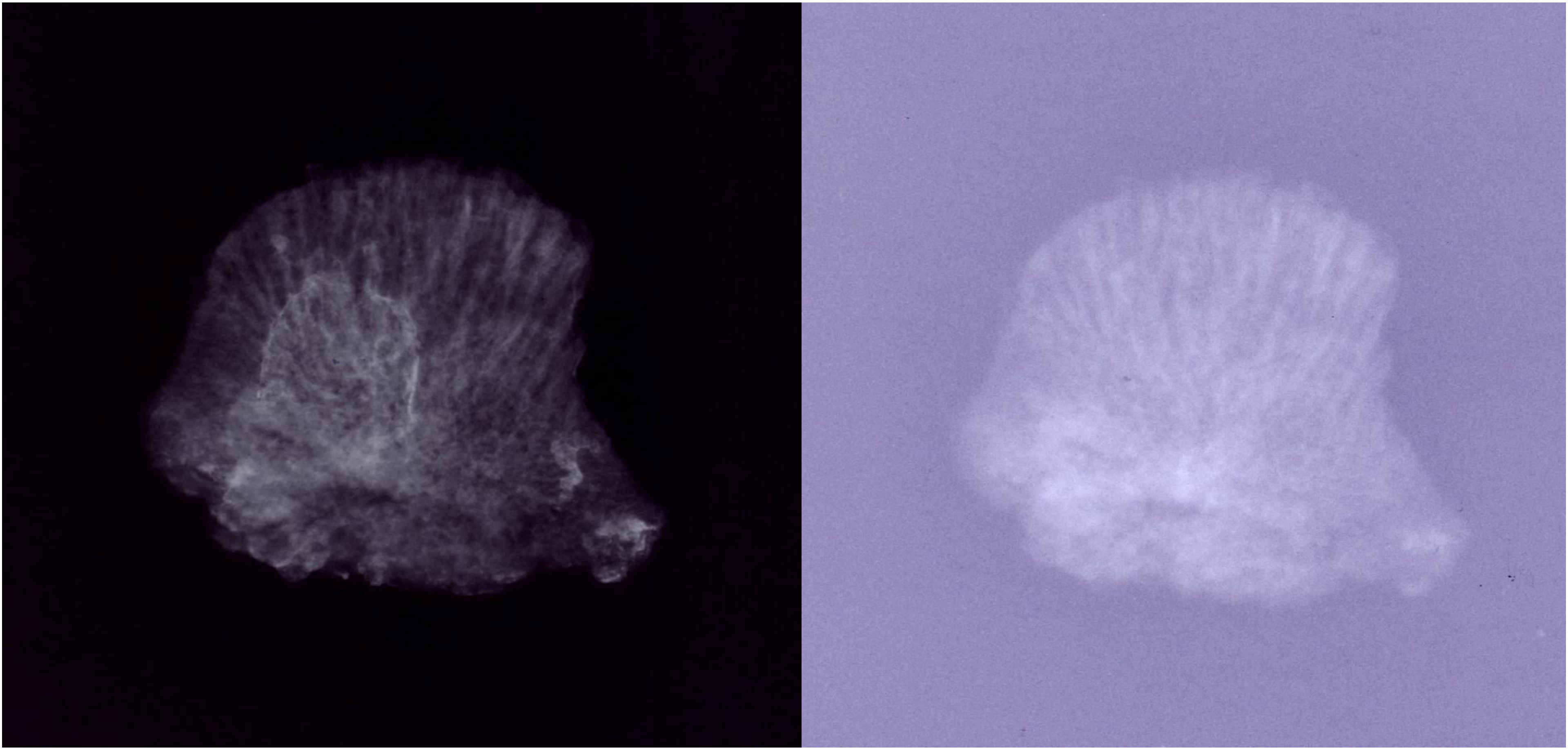

Figure 18. Images of specimen 3 isolated from the processed film images. In the dysprosium film (left) the artifact is clearly visible. A second artifact is also visible. Both artifacts are missing from the indium film (right). This indicates that the artifacts are comprised of a strong neutron absorber, such as Cd.

Conclusions

- Next steps:
  - Computed Neutron Tomography
  - Dissection and NAA on all six samples
  - Expand scope of study to include calciferous sponges and sea snails.
    - Research indicates Cd may show preferential disposition in calcium carbonate structures of sea snails and could be used to map time and exposure levels.

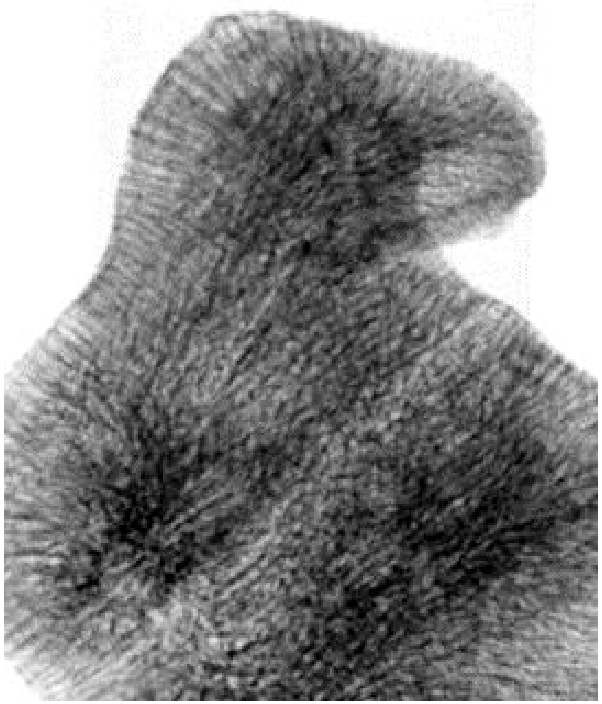

Figure 19. nCT of original specimen. Courtesy of FRM-II, Munich, Germany cold neutron beam (ANTARES). Captured at 20MW and 800L/D. Noise-filtered and compressed to 8-bit for video file.

Acknowledgements

- Sharlene Jolley (ISU)
- Dr. E. Nelson Eby (UML)
- Dr. Aaron Craft (INL)
- Glen Papaioannou (INL)
- The INL NRAD Reactor staff
  - Andrew Smolinski
  - Kyrone Riley
- Tyler Behrend (IFVEC)
- Idaho State University (ISU)
  - Department of Biological Sciences
    - Catherine Black
  - Chemistry Department
  - Reactor Staff
- The McClellan Nuclear Research Center (MNRC) staff
- The UMass-Lowell Research Reactor (UMLRR) staff
- Dr. Burhard Schilinger (TUM-ANTARES)
- The Technical University Munich FRM-II staff.

References

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Questions?