

Methods for deformity diagnostics: Radiography Kirsti Hjelde, Nofima •Sampling, fixation, labeling and sending of samples •General radiography knowledge and fish radiography in particular



Sampling:



Sample size depends on need for accuracy in the diagnostics and level of deformities. We commonly use 50-100 small fish or 30 large fish per group
Remember random sampling!
Preferably starved fish (if chemistry or live x-ray)

•Proper sedation and euthanasia to avoid gasping and movement in pictures

•Fresh fish always good for imaging!

Freezing



- Fish from 5 grams are easy to handle and send frozen
- First proper euthanasia, overdose of sedation or killing with a blow to the head
- Put the fish on the side on a smooth surface
 - Not directly on Styrofoam as they get stuck to it
 - Freeze them at -18 (normal freezer temperature) until properly frozen
 - Then pack them into plastic bags to avoid drying
- Big fish can be filleted to make transport easier and cheaper
 - Then the spine is either sent fresh on ice or frozen flat



Fixation

- Smaller fish (< 5g) can be preserved in 4% formaldehyde solution
 - Remember big enough glasses to avoid curvature of the body, and do not overfill them
 - Can be taken out of formalin and rinsed in ethanol, and then send in sealed plastic bags
 - 1 part fish to 10 parts of formalin is common for tissue samples for histology, but 1:5 works well for our purpose
- Formalin can give increased risk of cancer development, and is local irritating. Use gloves and work with good ventilation, preferably ventilation chamber

Labelling



- Single fish can be tagged with
 - pittag in the abdomen
 - notes stapled to the tail fin or put under the operculum
 - avoid putting notes in the mouth, as it forces the head up and the jaw open and thus affects the diagnostics
- Label groups on bag or box, with water proof marker pen or pencil
- If you send fish to someone else, be extremely careful to label groups properly
- It is better to write too much than not enough!









Wrapping

- Fish to be send by mail should be packed with absorbent material in waterproof boxes
- Remember the contamination danger for the receiver if there is disease in the fish group
- Fresh fish can be transported on ice
- Frozen fish need to be transported with freezing elements in the package or as a frozen sending

Sending

- Preferably over night or as a frozen package
- Inform the receiver when you send fish, and include information
 about the health status



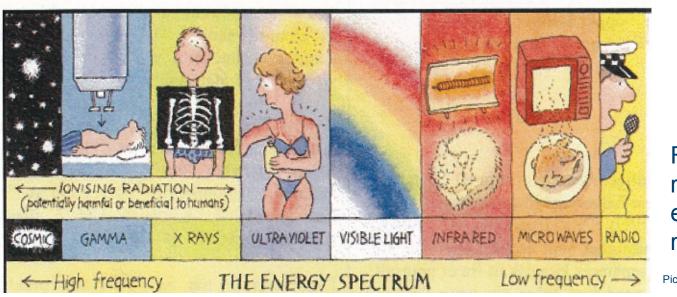


Sending over borders

- General principle: Sender is responsible for the transport being safe, and to get the proper health certificates for sending
- Sender needs to contact veterinary services to get health certificates and documentation on testing for several diseases (List 1 and 2 diseases)
- New regulations are less rigid, easier to send gutted and bled fish
- To Norway: Receiver uses Matilda VAM (Mattilsynet) to report expected sending
- Sending out of Norway in the EEC: Report to the TRACES system
- Contact the Food Authorities locally if questions about your particular sending

Radiography basic knowledge





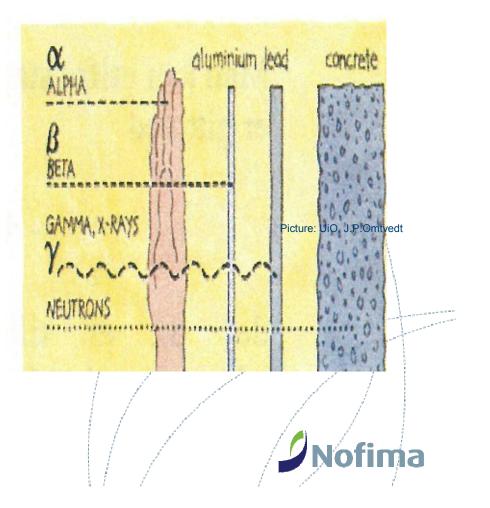
- Figure 1: Xrays are highenergy radiation
- Picture: UiO, J.P.Omtvedt

- Discovered in 1895 by Wilhelm C. Röntgen
- Widely used in medicine for diagnostic purposes
- X-rays are high-energy electromagnetic radiation created by rapid loss of speed of the electrons inside the x-ray source



 Due to their energy, x-rays penetrate soft tissues such as muscle and intestines

 Bone, lead and other hard substances, as well as water, absorb the radiation





•The image is a result of the number of high-energy x-rays being able to penetrate the different tissues

•Hard materials absorb a lot of x-rays, leading to few reaching the film and a whiter area in the image. Black areas in a picture have received a lot of x-rays and thus have lower tissue density

•X-rays loose their energy when absorbed, and they never remain in the object after radiation

•As soon as you turn the x-ray source off, the radiation is gone

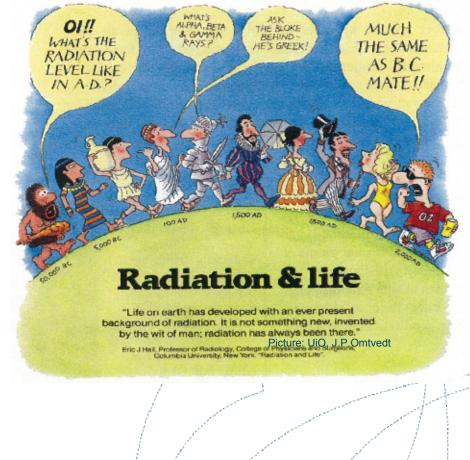


Picture: Wikipedia

Radiation security

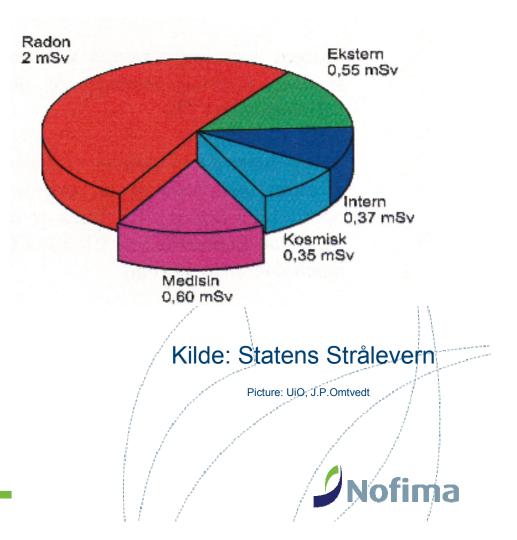


- Normal use of radiography is not dangerous to the person performing the examination. BUT...
- Good routines are important to prevent injury and to make people feel safe at work
- All radiation is potentially dangerous, as there is no "Safe zero-level" for exposure





- Mean normal radiation exposure for a Norwegian per year:
- Radon gas is by far the most important source for radiation
- Potential danger from medical radiation far less than the benefits





- Radiation decreases exponentially through the tissue
- X-rays are potentially harmful by creating very reactive radicals that can harm the DNA or RNA
- Cell that are in division processes are most vulnerable for DNA/RNA damage
- DNA-damage is usually repaired by the normal repairing systems in the body



- We separate between
 - damage to body cells, giving a possibility of cancer
 - damage of germ cells (sperm and eggs) giving a possibility of maldevelopment of a fetus
- Particular areas to protect are genitals, thyroid gland and growth zones (children)
- Scattered rays can appear around the object

Easy routines for safe radiography work!

- Keep distance to the object if possible
- Lead in walls of radiography room protects the surroundings and is prohibited by Norwegian regulations
- Lead clothing for protection, especially neck and hips
- Dosimeters for measuring personal doses of radiation, checked routinely every two months
- Low exposure dose if possible, limit number of exposures
- Never let pregnant women or children in radiography rooms unnecessary



Lead apron for gonadal protection for children Picture: Euli catalogue 2005



Dosimeter used by Statens Strålevern

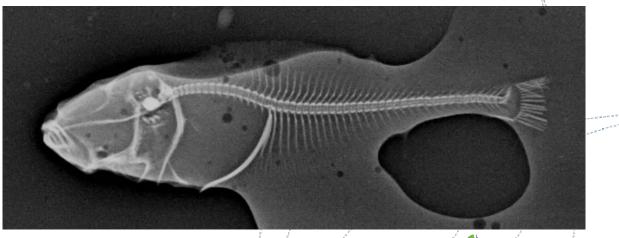
Picture: UiO, J.P.Omtvedt

Fish radiography



- What is special about fish radiography?
- Fish vertebrae are very small compared to even the small bones in a kitten's paw
- The vertebral length in a 8 cm long salmon will be about 1 mm
- Thus, there is a need for sensitive equipment with the possibility of using low exposure levels
- Human mammography equipment is especially designed for detecting micro calcifications in soft tissue
- And because of that a good tool for small fish (<200 grams radiography!

Digital mammography image of a 0,8 g halibut larvae laying in methanol



Analogue radiography



- Diagnostic tool for more than a hundred years
- Film-foil system where light-sensitive film is exposed for x-rays, and developed in a dark room
- Detail level limited from type of film, exposure time and radiation strength (adjustments in mAs and kV)
- mAs = strength in cathode X seconds
- kV = kilovolt = radiation energy
- Low dosage and long exposure time give longer greyscale and more detailed pictures
- High dosage and short exposure time give higher contrast (short greyscale)



Digital radiography

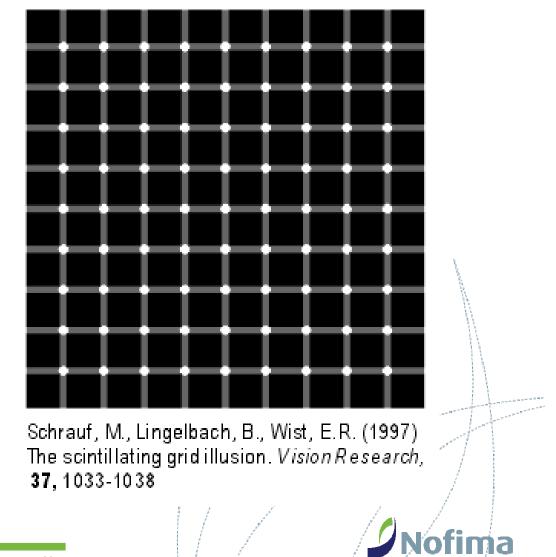
- Same x-ray sources as analogue radiography
- Reusable films in semi digital systems
- No film in direct digital radiography, picture-plate system in semidigital radiography
- Benefits:
 - Preset menus for improved picture quality
 - Lower doses for same result
 - Easy picture storage
 - Easy picture handling
- Drawbacks
 - Limit in pixel quality when looking at details
 - Possibility of artifacts made by picture treatment in the computer (contrast enhancement, varied greyscale)





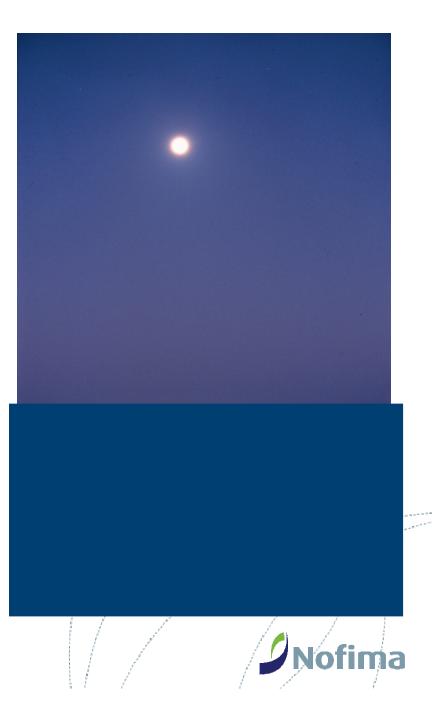
Part 2: Understanding the picture

- Count the black dots in the grid!
- "You see what you look for, you recognize what you know"



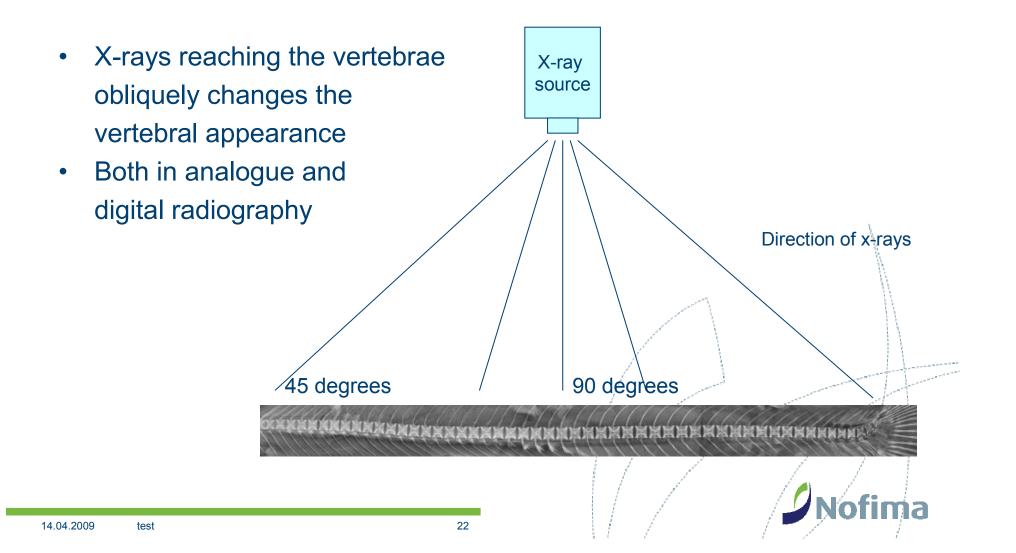
•The contrast phenomenon: Note the darker zone between the moon and the sea reflection.

The same thing happens when we look at black-white contrasts, we tend to increase the contrast subconsciously.

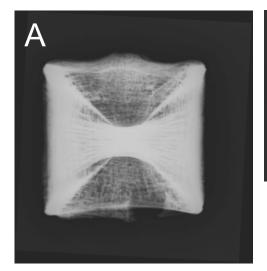




Common artefacts in fish radiography: Oblique beam direction



"Narrow vertebrae" caused by x-ray direction:





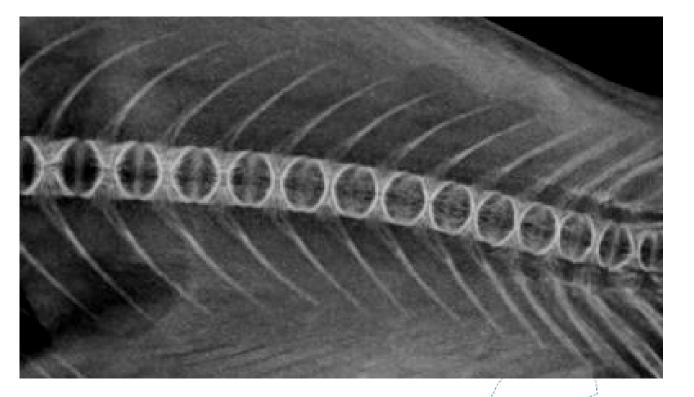


Same single vertebra taken in two different beam directions.

- In A the direction of the x-rays were 90 degrees to the vertebra
- •In B there was a 45 degrees angle
- •C is the same as B, but with an less light and higher contrast, to show the double €-appearance better



"String of pearls"-appearance/Ring vertebrae



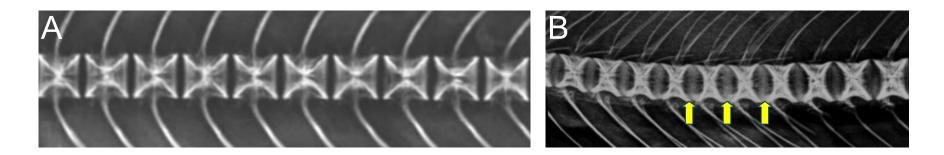
• Caused by oblique beam direction

14.04.2009 test

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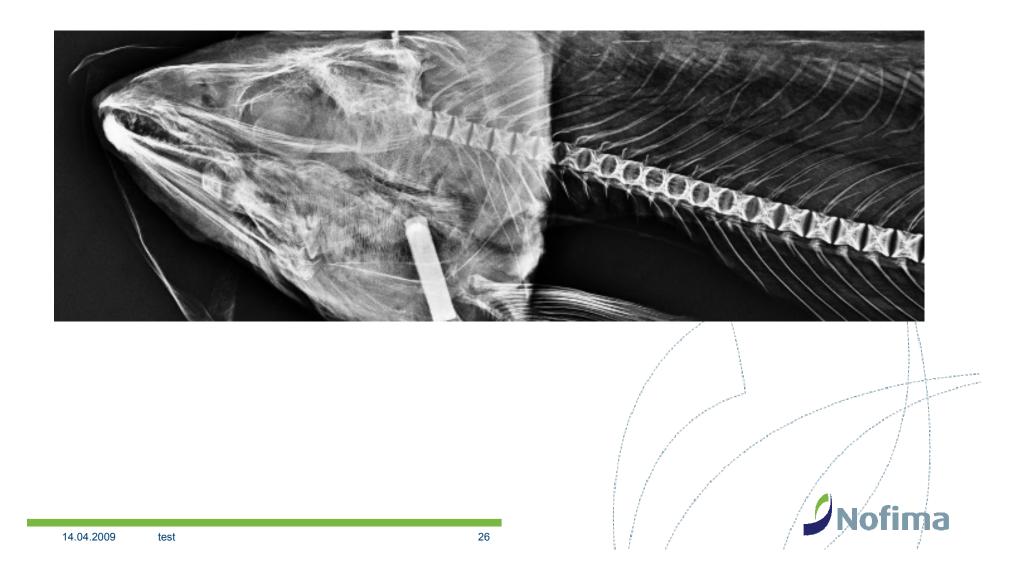
"White intervertebral lines"



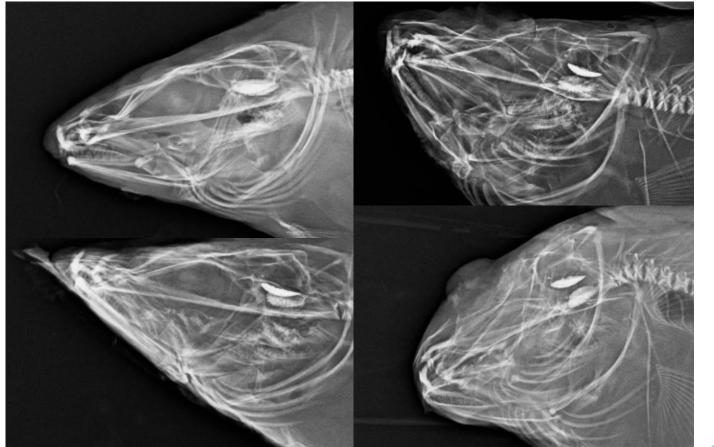
- A is a normal salmon spine with regular vertebrae and no intervertebral tissue
- B is the same spine in an oblique angle to the x-ray source, giving an impression of intervertebral tissue (white lines) and an irregular, narrow vertebral appearance. The contrast is also higher than in picture A.
- This phenomenon occurs when the x-ray angle is oblique or when the fish is not laying straight (poor packaging, rigor mortis in a curved position)
- Digital radiography may enhance this artifact by using contrast enhancement in the picture treatment

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Oblique beam angle:



Position artefacts

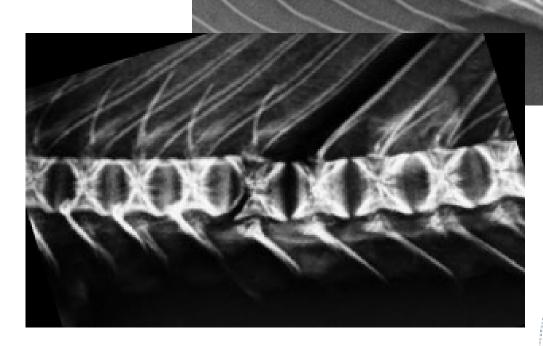


- Cod head frozen in different positions,
- Normal to the top left
- Note the otoliths

Nofima

Handling artefacts

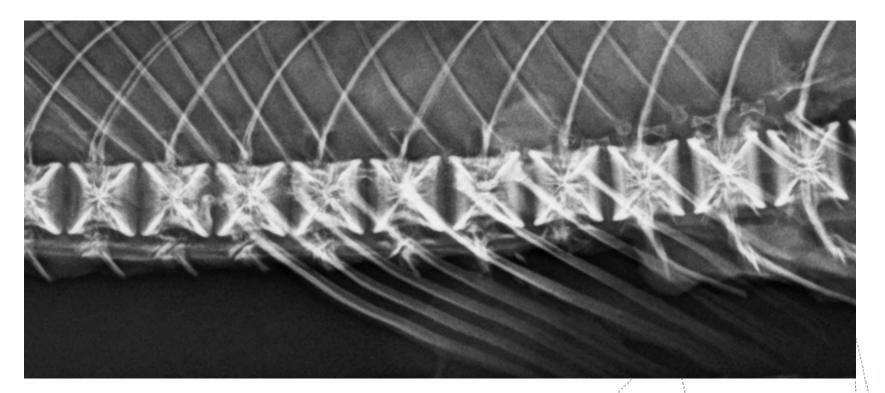
"axial deviation" caused by filleting



•"Fillet knife disease", vertebral deformity caused by rough filleting



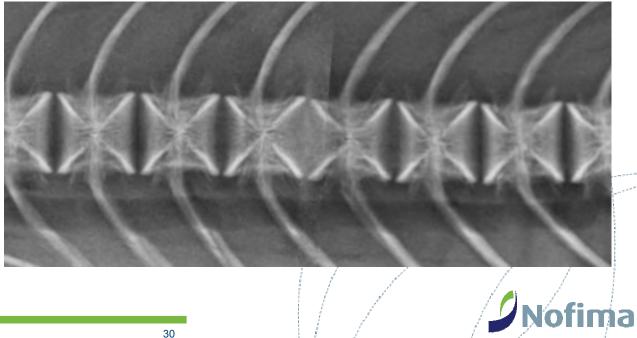
Overlapping structures



• Ribs overlapping the vertebrae of a large salmon

Artefacts in digital images:

- Stitched image artefact ٠
- Stitching is used in large fish to get the entire fish • in one picture (human scoliosis cassettes)



Artefacts in digital images: Contrast adjustments

Osteopenia, poorly mineralised vertebrae in salmon

•Normal image on top

•Increased contrast in the middle

•Decreased contrast in the bottom image

