ELEMENTARY, MY DEAR WATSON! BIOMEDICAL IMAGING TECHNIQUES FOR FORENSIC ANALYSES OF TRAUMA, DEFECTS, AND DISEASE IN MARINE MAMMALS

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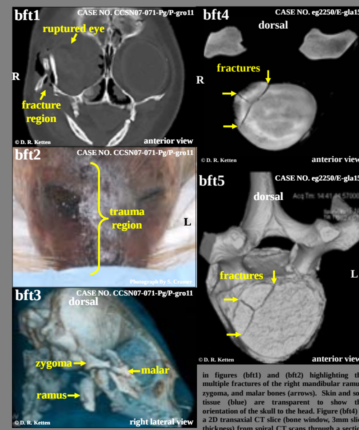
ABSTRACT Over the last decade, there has been a rapid increase in the use of biomedical imaging procedures on both live and post mortem marine mammals. With the advent of larger bore machines, more rapid data acquisition, and increased weight capacities, computerized tomography (CT) and magnetic resonance imaging (MRI) became more accessible and more useful diagnostic and research tools. Scanners are now capable of acquiring data in high resolution as fast as 10 mm/sec, providing a table time of less than 5 minutes for a full body scan of a 1.5 meter animal. In addition, ultra-high resolution images effectively produce micro-imaging at 100 micron sectioning through post-scan processing with most modern clinical units. Because of the rapidity of modern imaging techniques, it is feasible to perform *in vivo* scans of many marine mammal species at micro and macro sectioning including larger specimens such as heads of juvenile mysticetes. This poster will present an overview of CT and MRI scan protocols that have been developed over the last decade for imaging marine mammals as well as modifications of those protocols to maximize detection and diagnosis of the following categories of pathologies in both cetaceans and pinnipeds; blunt force trauma, explosive trauma, fractures, gunshot wounds, pneumothorax, pneumonia, hemorrhage, brain lesions, hearing pathology, parasites, emboli, and disease. Explicit case histories will be employed to demonstrate each condition.

INTRODUCTION Spiral computed tomography (CT) is an x-ray tomographic technique in which an x-ray beam, in the shape of a thin fan, rotates and passes through thin axial (sometimes called transverse or transaxial) sections of a patient from various directions and is captured by detectors that measure the intensity of the attenuated radiation as it emerges from the body (Prokop, 2003). These attenuation coefficients are converted into "CT numbers" also known as "Hounsfield Units" which are then converted into shades of gray that are displayed as an image (Prokop, 2003). Successful use of CT in the experimental and anatomical investigation of marine mammals has been reported in the clinical literature since the early 1980's (Nordoy and Blix, 1985; Ketten and Wartzok, 1990; Luo and Ketten, 1991; Hillman, 1991; Woodhouse and Rennie, 1991; Ponganis et al., 1992; Endo et al., 1999; Ketten and Montie, 2007, Montie, 2006, Arruda et al., 2007); several years after it's first use at the Mayo Clinic in 1973 (Van Bonn, Jensen, and Brook, 2001). Successful CT scans of marine mammals have also been recorded in clinical environments (Haulena et al., 1998; Van Bonn et al., 2001; Williams and Dunnigan, 2005). However, imaging protocols available for use in marine mammal studies were dedicated primarily to research applications (Van Bonn et al., 2001). Clinical methods had not been standardised to the extent they had in domestic animal species (Van Bonn et al., 2001) and techniques used in most clinical cases were likely derived from human or domestic species protocols. Additionally, most if not all clinical CT scanners have integrated software limitations designed to control radiation dose and exposure times. These limitations are generally not a factor when scanning smaller samples. But in cases of larger more massive specimens, limitations on parameters can greatly effect image resolution to a point where diagnostics are not possible (Prokop, 2003). Larger and more massive tissues such as juvenile mysticete heads or larger whale odontocetes often require higher dose levels and longer exposure times to achieve the proper tissue penetration and image resolution necessary for accurate analysis and diagnoses. We propose techniques and protocols can be developed for use in CT/MRI imaging of both small and large marine mammals using standard techniques that fall within the range of clinical CT/MRI scanner limitations and techniques developed to achieve proper tissue penetration in larger more massive animals. Our objective is to establish standards for biomedical CT and MRI imaging techniques applicable to diagnostic imaging, forensics, and research studies of both live and post mortem marine mammals.

MATERIALS & METHODS Marine mammal samples used in this research were obtained in accordance with state and federal regulations listed under the Marine Mammal Protection Act (MMPA) of 1972 as amended. Animals were obtained from local stranding networks, fisheries services, aquariums, and marine mammal rehabilitation centers. Live animals were scanned under the observation and guidance of licensed veterinarians and research technicians. Nearly 600 specimens (57 species, within 17 families, and 3 orders including Cetacea, Pinnipedia, and Sirenia) were scanned over the last decade. Select cases considered unique in terms of imaging techniques used and pathologies observed are presented here. CT scans were acquired at the Computerized Scanning and Imaging Facility (CSI) of Woods Hole Oceanographic Institution (WHOI), Woods Hole, MA and Massachusetts Eye & Ear Infirmary (MEEI), Boston, MA, using Siemens® Volume Zoom Ultra-High Resolution (UHR) computed tomography (CT) systems for volume scanning. Animals were scanned craniocaudal, prone position, whole, and *in vivo* when possible or were sectioned by means of dissection and scanned within 24 hours post mortem using ultra-high resolution spiral submillimeter protocols for continuous data acquisition. Effective mAs and KV were within the range of 2-300 mAs/80-120 KV for smaller animals and 3-700 mAs/120-140 KV for larger specimens. Transaxial slice increments were typically set at 8 mm slice thicknesses through whole animals, 3 mm through head sections and 0.5/1.0 mm through sections of the skull that included the ears. Image reformats were done at slice increments ranging from 0.1 mm to 10 mm (Ketten and Montie, 2007). Shaded Surface Display (SSD), Volume Rendering Technique (VRT), and target tissue segmentation 3D graphics were reconstructed from tissue attenuation data using 3D visualization software applications.

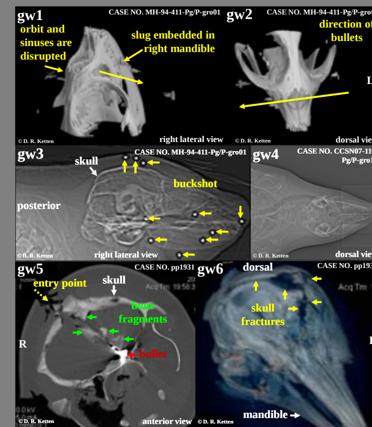
RESULTS

BLUNT FORCE TRAUMA



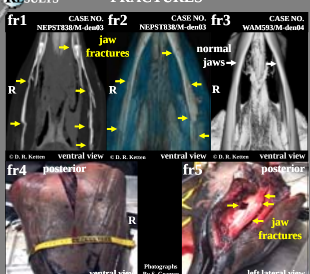
FIGURES BFT1 – BFT5. Figure (bft1) is a 2D transaxial CT slice (bone window, 3mm slice thickness) from spiral CT scans through the head of a post mortem harp seal (*Phoca groenlandica*) with multiple fractures of the right mandible, orbit, and a rupture of the right eyeball. Figure (bft2) is a photograph of the wounded seal prior to CT scanning. Note the edema and ecchymosis around the right mandible and eye and a displacement of vitreous humour from the right eye socket. Figure (bft3) is a 3D Volume Rendering Technique (VRT) reconstruction, 1 mm slice thickness from 3 mm spiral CT scans, of the head of the seal in figures (bft1) and (bft2) highlighting the multiple fractures of the right mandibular ramus, zygoma, and malar bones (arrows). Skin and soft tissue (blue) are transparent to show the orientation of the skull to the head. Figure (bft4) is a 2D transaxial CT slice (bone window, 3mm slice thickness) from spiral CT scans through a section of vertebra of a post mortem northern right whale (*Eubalaena glacialis*) with a dorso-lateral transverse t-shaped vertebral stress fracture (arrows) of the anterior thoracic vertebral facet. The fracture spans from the superior medial margin to the inferior lateral margin with a dorso-lateral to medial intersection. Figure (bft5) is a 3D Shaded Surface Display (SSD) reconstruction, 1 mm slice thickness from 3 mm spiral CT scans, of the vertebra shown in figure (bft4). (Specimens courtesy of New England Aquarium - (NEAq) and the Cape Cod Stranding Network - (CCSN))

GUNSHOT WOUND

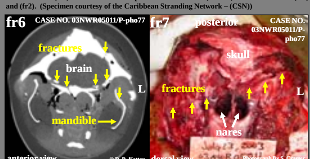


FIGURES GW1 – GW4. Figures (gw1) and (gw2) are 3D Shaded Surface Display (SSD) reconstructions, 1 mm slice thickness from 3 mm spiral CT scans, of a harp seal (*Phoca groenlandica*) mortally wounded by bullets from a shotgun blast. Figue (gw1) shows the bone fragments displaced by the bullets. Figure (gw2) shows the direction of the bullets through the skull. Figure (gw3) is a 2D sagittal topogram of the mortally wounded seal showing the resulting bullet pattern (bright white dots) within the head. Figure (gw4) is a 2D coronal topogram of a normal harp seal (*Phoca groenlandica*). Figure (gw5) is a 2D transaxial CT slice (bone window, 3 mm slice thickness) from spiral CT scans through the head of a harbor porpoise (*Phocoena phocoena*) mortally wounded by a bullet wound to the head (red arrow). Note the bullet path (yellow arrow) and resulting bone fragmentation (green arrows). Figure (gw6) is a 3D Volume Rendering Technique (VRT) reconstruction, 3 mm slice thickness from 3 mm spiral CT scans, of the post mortem porpoise in figure (gw5). Skin and soft tissue (blue) are transparent to show the orientation of the skull to the head. (Specimens courtesy of Dr. Susanne Prahl, University of Kiel, National Marine Fisheries Service - (NMFS) and, New England Aquarium – (NEAq))

FRACTURES

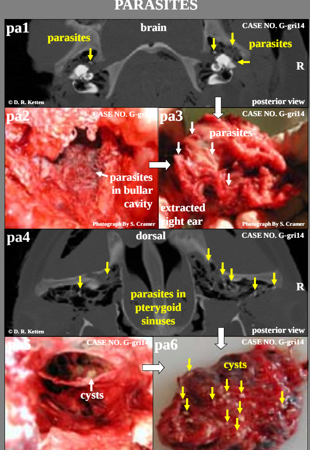


FIGURES FR1 – FR5. Figure (fr1) is a 2D coronal CT slice (bone window, 3 mm slice thickness) from spiral CT scans through the head of a post mortem blainville's beaked whale (*Mesoplodon densirostris*) with bilateral compound mandibular fractures (arrows) (Ketten, 2002). Figure (fr2) is a 3D Volume Rendering Technique (VRT) reconstruction, 1 mm slice thickness from 3 mm spiral CT scans, of the head shown in figure (fr1) highlighting the fractures. Skin and soft tissue (blue) are transparent to show the orientation of the skull to the head. Figure (fr3) is a 3D Shaded Surface Display (SSD) reconstruction, 1 mm slice thickness from 3 mm spiral CT scans, of the head of a post mortem blainville's beaked whale (*Mesoplodon densirostris*) with normal jaws. Figure (fr4) shows patterns of bruising along the exterior ventral surface of the head. Tissues were removed during dissection which exposed the mandibles (fr5) and confirmed the presence of fractures (arrows) seen in CT scans (fr1) and (fr2). (Specimen courtesy of the Caribbean Stranding Network – (CSN))



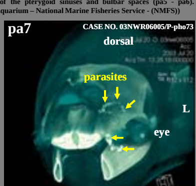
FIGURES FR6 – FR7. Figure (fr6) is a 2D transaxial CT slice (soft tissue window, 3 mm slice thickness) from spiral CT scans through the head of a post mortem harbor porpoise (*Phocoena phocoena*) with multiple longitudinal and comminuted fractures of the skull and left mandible (arrows). Figure (fr7) is a photograph of the porpoise shown in figure (fr6) during dissection. Note the transverse fracture across the skull (yellow arrows). The etiology of this injury is unknown. (Specimen courtesy of the National Oceanic and Atmospheric Administration – (NOAA))

PARASITES

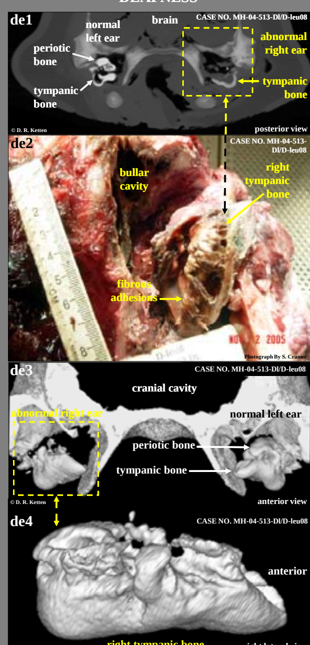


FIGURES PA1 – PA6. Figure (pa1) is a 2D transaxial CT slice (bone window, 0.5 mm slice thickness) from spiral CT scans through the head of a post mortem risso's dolphin (*Grampus griseus*) with a parasitic infestation (arrows). Post mortem extraction of the right ear (pa2 - pa3) confirmed the presence of a conglomerate of nematode parasites and cysts (arrows) seen in CT scans (pa1) invading the inferior and posterior bullar spaces (pa2). Figure (pa4) is a second 2D transaxial CT slice (bone window, 0.5 mm slice thickness) from spiral CT scans through the head of the post mortem risso's dolphin (*Grampus griseus*) seen in figure (pa1). The presence of numerous ovoid cysts (arrows) observed in CT scans (pa4) were confirmed during post mortem dissection of the pterygoid sinuses and bulbar spaces (pa5 - pa6). (Specimen courtesy Woods Hole Aquarium – National Marine Fisheries Service - (NMFS))

FIGURE PA7. Figure (pa7) is a 3D Volume Rendering Technique (VRT) reconstruction, 1 mm slice thickness from 3 mm spiral CT scans, of the head of a harbor porpoise (*Phocoena phocoena*) with a parasite infestation of the blubber, muscle, and melon (arrows) dorsal and anterior of the left eye. Skin and soft tissue (blue) are transparent to show the orientation of the skull to the head and of the parasites to the surrounding tissues. (Specimen courtesy of National Oceanic and Atmospheric Administration – (NOAA))

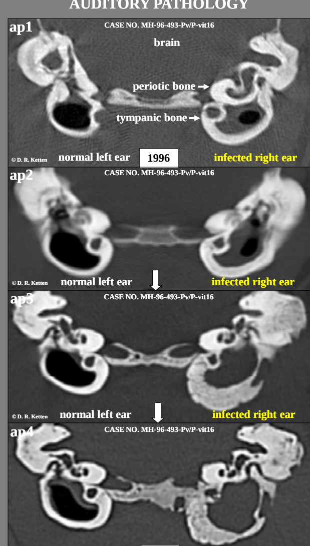


DEAFNESS



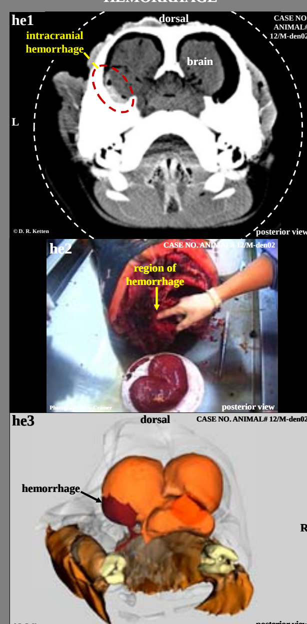
FIGURES DE1 – DE4. Figure (de1) is a 2D transaxial CT slice (bone window, 0.5 mm slice thickness) from spiral CT scans of the head of a post mortem beluga whale (*Delphinapterus leucas*) with an anomaly of the right ear (Ketten, 2005b). Note the absence of a right periotic bone. Figure (de2) shows the right tympanic bone after extraction. Figures (de3) and (de4) are 3D Shaded Surface Display (SSD) reconstructions, 0.5 mm slice thickness from 1 mm spiral CT scans, of the head of the post mortem beluga whale (*Delphinapterus leucas*) shown in figure (de1) highlighting the right and left ear bulla in relation to the skull (de3) and the abnormal right ear (de4). (Specimen courtesy of New England Aquarium - (NEAq))

AUDITORY PATHOLOGY



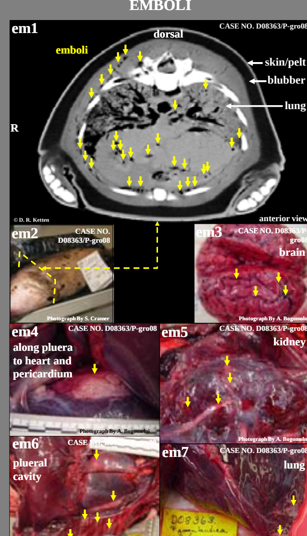
FIGURES AP1 – AP4. Figures (ap1) through ap4) are 2D transaxial CT slices (bone window, 0.5 mm slice thickness) (acquired from 1996 to 2002) from spiral CT scans through the head of a live captive harbor seal (*Phoca vitulina*) showing the progression of a chronic middle ear infection of the right ear (Williams and Dunnigan, 2005) . Note the deformity and sclerosis around the right tympanic and periotic bones. The left temporal bone of this animal is normal. (Specimen courtesy of the Woods Hole Aquarium – National Marine Fisheries Service - (NMFS))

HEMORRHAGE



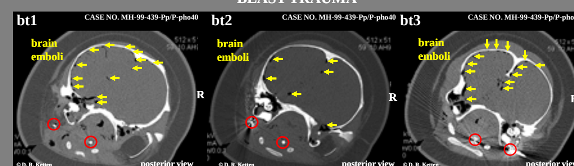
FIGURES HE1 – HE3. Figure (he1) is a 2D transaxial CT slice (soft tissue window, 3 mm slice thickness) from spiral CT scans through the head of a blainville's beaked whale (*Mesoplodon densirostris*) with an intracranial hemorrhage (Ketten, 2005a) (red dashed circle) dorso-lateral to the left cerebral hemisphere in the left temporal fossa. The dashed white line shows a region of soft tissue removed prior to scanning. Dissection and removal of the brain (he2) confirmed the presence of the hemorrhage observed in CT scans shown in figure (he1). Figure (he3) is a 3D target tissue segmentation reconstruction, 1 mm slice thickness from 3 mm spiral CT scans, of the head shown above in figures (he1) and (he2) using 3D Slicer® visualization software. Shown are the positions of the hemorrhage (red), the brain (orange), the ear bulla (beige), and the jaw fats (bronze). The skull is transparent to show the position of the anatomy relative to the skull. (Specimen courtesy of the Caribbean Stranding Network – (CSN))

EMBOLI



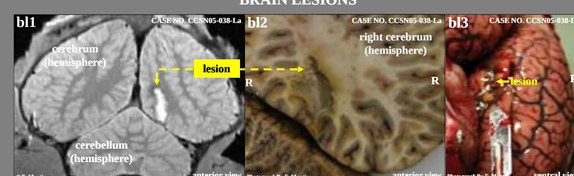
FIGURES EM1 – EM7. Figure (em1) is a 2D transaxial CT slice (soft tissue window, 3 mm slice thickness) from spiral CT scans through the thoracic cavity of a harp seal (*Phoca groenlandica*) drowned subsequent to entanglement in a commercial fisheries gillnet (NOAA/NMFS, 2007, personal communication). The corresponding photograph (em2) shows the position of the transaxial slice in figure (em1). Gas bubbles were observed (arrows) in blood vessels, muscle, lungs, heart, brain and kidneys (em3 - em7) during dissection (Bogomolni, 2007). (Specimen courtesy of National Oceanic and Atmospheric Administration - (NOAA) and National Marine Fisheries Service - (NMFS))

BLAST TRAUMA



FIGURES BT1 – BT3. Figures (bt1 – bt3) are 2D transaxial CT slices (soft tissue windows, 3 mm slice thickness) from spiral CT scans through sections of the head of a harbor porpoise (*Phocoena phocoena*) exposed to an underwater explosion post mortem. Arrows point to multiple air emboli observed in the brain. Implanted pressure gages are indicated by red circles. (Specimen courtesy of New England Aquarium – (NEAq) and Cape Cod Stranding Network – (CCSN))

BRAIN LESIONS



FIGURES BL1 – BL3. Figure (bl1) is a 2D transaxial magnetic resonance Image (MRI) slice through the brain of a post mortem adult atlantic white-sided dolphin (*Lagenorhynchus acutus*) that shows a lesion in the right cerebral lobe of the right cerebral hemisphere (bl2) (Montie, 2006). Extraction of the brain (bl3) confirmed the presence of the lesion shown in figure (bl1). (Specimen and images courtesy of E. Montie – University of South Florida - (USF).

DISCUSSION **FRACTURES** - Observation of the jaws during gross dissection confirmed the multiple bilateral mandibular fractures (Ketten, 2005a) seen in CT scans. The bruising on the ventral surface of the head may be indicative of blunt force trauma. The etiology of the fractures seen in the harbor porpoise (*Phocoena phocoena*) is unknown. Additional observations of multiple cranial contusions and a homogenous brain in CT scans suggest the mechanism(s) was consistent with blunt or blast trauma resulting in immediate death (Norman, 2004). Determination of these events being pre or post mortem is contingent upon evidence presented in histological findings. **PARASITES** - The parasites found in the risso's dolphin (*grampus griseus*) during dissection were later confirmed as two species of *Metastrongyles spp.* (T.P. Lipscomb, Armed Forces Institute of Pathology (AFIP), Department of Pathology, Washington, D.C.). Using CT, Zucca et al., (2004) also reported findings of *Crassicauda grampicola* infestation in the pterygoid sinuses and peribullar regions of a risso's dolphin (*Grampus griseus*). Dissection confirmed the presence of parasites observed in the harbor porpoise during CT scanning. Further microscopic and histologic analysis will be used to identify the nematode species. **DEAFNESS** - The absence of a right periotic bone in this beluga whale (*Delphinapterus leucas*) is likely due to infection and necrosis of bone and surrounding tissues (Ketten, 2005b). Other possibilities considered were parasite infestation, trauma, or congenital defect. The term of infection was not known. The condition of the ear during dissection indicated the animal was likely deaf in the right ear. The extraction of the right ear showed complete disintegration of the periotic bone and a rugose, pitted and convoluted tympanic bone with extensive radiating adhesions fibrous in nature and a peribullar cavity filled with a dense black granular material (Ketten, 2005b). The left ear was normal. Final diagnosis indicated the cause of death was due to natural causes, however diagnosis of the right ear pathology remains an unknown etiology until further histological analysis can be performed. **AUDITORY PATHOLOGY** - This captive harbor seal (*Phoca vitulina*) was brought to us in 1996 with an infection of the middle ear. CT scans and analysis of cultures obtained from the infected ear indicated *chronic suppurative otitis media* (Williams and Dunnigan, 2005). The seal was placed on a treatment regime and CT scanned several times between 1996 and 2002 to monitor the osteomyelitic process in the right temporal bone and basisphenoid. **HEMORRHAGE** - The etiology of this hemorrhage is unknown. We present this case to emphasize the efficacy of CT to delineate soft tissue(s) at a resolution quality needed for accurate 3D reconstruction. **EMBOLI** - This harp seal (*Phoca groenlandica*) was by-caught in a commercial fishing vessel net. Data related to the seals depth when entangled, duration of entanglement, and rate of ascent were not available. The seal was scanned within several hours of capture. During dissection no anaerobic organisms were isolated in the thorax or the abdomen (Bogomolni, 2007). Confirmation that this event is consistent with emboli seen in decompression sickness or "the bends" is contingent upon further histological analysis and a better understanding of marine mammal dive physiology. Here the advantage of scanning tissues *in situ* prior to dissection is the prevention of false emboli sometimes caused by the introduction of air bubbles into cavities, vessels, or tissues exposed to ambient air pressures when incised. **BLUNT FORCE TRAUMA** – This harp seal (*Phoca groenlandica*) showed external evidence of trauma to the head; a ruptured eye, swelling, and contusion. CT scans confirmed underlying fractures to the skull and mandible, the pattern of which is consistent with blunt force trauma. The vertebra is from a northern right whale (*Eubalaena glacialis*) found in a moderate stage of decomposition with a large wound along the axis of the back (Hamilton, 1995). The stress of severe bending of the back to avoid collision with a ship is the likely mechanism in the vertebral fracture due to the magnitude of the breaks in such dense bone (Hamilton, 1995). **GUNSHOT WOUND** - The bullets shown in figure (gw1) are pellets from a shotgun blast that was fired at a slight angle just above the head. Bone fragments and injury patterns observed in the 3D reconstructions allowed us to determine the path of the bullets through the skull. The blast came from the left lateral side of the head and passed through to the right lateral side. The porpoise was brought to us from Germany where it had been mortally wounded by a single shot to the head. The displacement of bone into the cranium suggests the entry point was on the dorsal surface of the head. In both cases the evidence gathered from CT data could prove valuable in state or federal courts should legal action be pursued. **BLAST TRAUMA** – This harbor porpoise (*Phocoena phocoena*) was fully submerged in a test pond (at a predetermined distance from the charge) and exposed to a 300 psi underwater blast post mortem. Using both CT and gross dissection we observed injuries consistent with underwater primary blast injury (UPBI) (brain emboli) that indicated this event would likely have been fatal (Ketten et al., 1999; Ketten et al., 2005; Phillips, 1991). **BRAIN LESION** - MRI revealed two common dolphins (*Delphinus delphis*) and two atlantic white-sided dolphins (*Lagenorhynchus acutus*) contained brain lesions (i.e. 4 out of 16 dolphins randomly imaged contained a brain lesion) (Montie, 2006) and a heavy infestation of the nematode *Stenurus spp.* in the cranial and otic sinuses. In at least three cases, histological findings suggest parasite migration as the causative agent. However, no adults of ova were observed in the brain. (Montie, 2006).

CONCLUSION The use of CT and MRI technology as a non-invasive, fast, and accurate means of collecting research and clinical data is gaining popularity. Used in conjunction with gross observation, dissection, and histological analysis, the technology gives us the ability to observe anatomy *in situ* at a very high resolution and accurately make clinical and research diagnoses. Development of new techniques and refinement of existing techniques will continue. Through these developments we hope to contribute to both research and clinical communities, standards for biomedical CT and MRI imaging techniques applicable to diagnostic imaging, forensics, and research studies of both live and post mortem marine mammals.

LITERATURE CITED Full citations are appended to abstract.

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Case Images: http://www.whoi.edu/csi