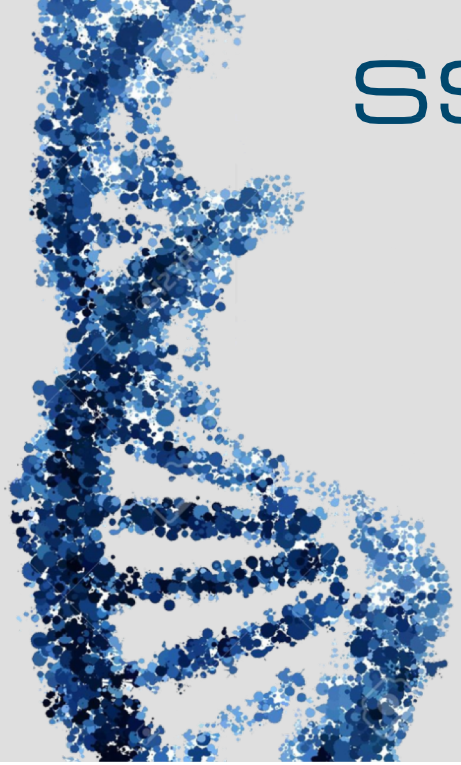


SSEF

The Pearl Symposium, Bahrain I November 2019

NEW FRONTIERS IN PEARL ANALYSIS: Age Dating, DNA Fingerprinting, and novel Radiographic Methods

Presentation by Dr. Michael S. Krzemnicki, SSEF

with Dr. Laurent E. Cartier, Dr. Bertalan Lendvay, Dr. Nadja Morf, Dr. Irka Hajdas, Dr. Vincent Revol, Carina Hanser.

INTRODUCTION

"[Pearls] occupy the first rank…and the very highest position among valuables."

Pliny the Elder, Historia Naturalis

Main duty of gemmological laboratories in pearl testing:

- **Separation of natural pearls from cultured pearls**

Natural Pearl pendant of Marie Antoinette (1755-1793)
sold for US$ 36 million
at Sotheby's Geneva in November 2018

Cultured Pearl pendant
offered for US$ 25
on internet (Alibaba) in November 2019

INTRODUCTION

Although in some cases very straightforward and easy, the separation of natural pearls from cultured pearls sometimes may also be very difficult and challenging.

The conclusion of pearl testing (natural vs. cultured pearl) is always based on an **educated interpretation** of observed and analysed data pertaining to that pearl.

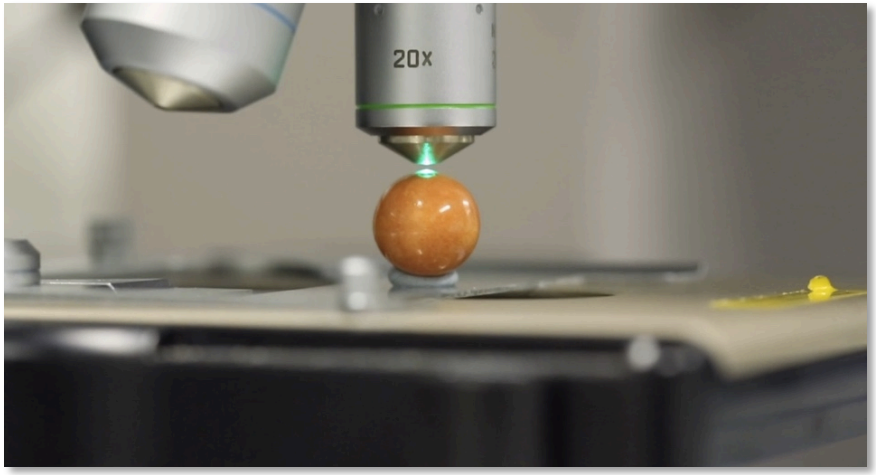
As such, any pearl testing conclusion is always an **expert opinion.**

The Peregrina pearl, tested at SSEF.

INTRODUCTION

Pearl testing is always based on a **combination of various testing methods**, such as:

External observation (e.g. microscopy), visualisation of internal structures (e.g. X-ray radiography), chemical analyses (e.g. ED-XRF), structural analyses (e.g. Raman spectroscopy), to name a few.



Novel methods are evaluated on a continuous basis to get new insights into pearl formation and their internal structures, and finally with the aim to support pearl testing as a client service to the trade and consumers.

RADIOCARBON DATING OF PEARLS

Age dating based on the decay of radiocarbon ^{14}C.

This method is able to provide supporting evidence for historic pearls, and may also assist in natural vs. cultured pearl separation.

RADIOCARBON CYCLE

MICADAS Mini Carbon Dating System (Accelerator Mass Spectrometer). Image ETH Zurich & ionplus AG

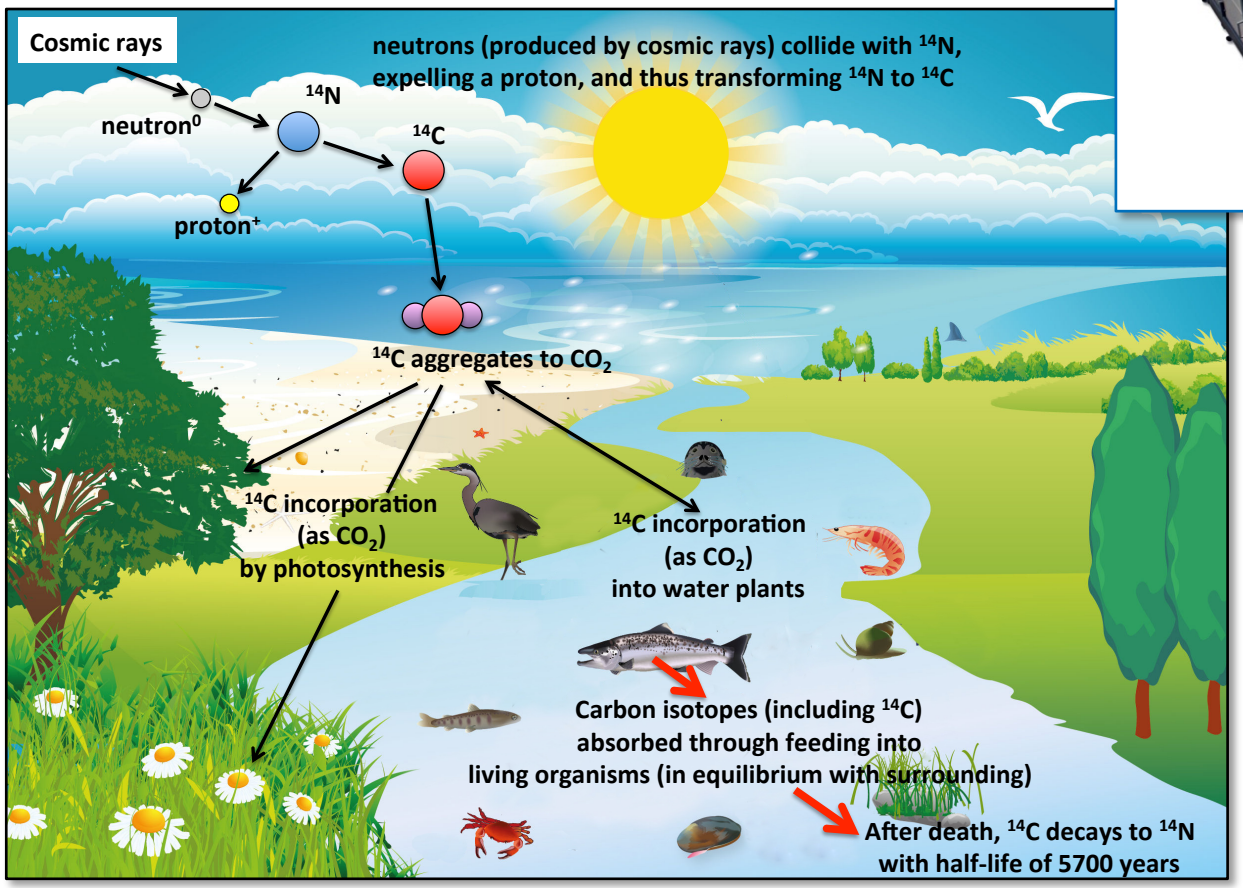
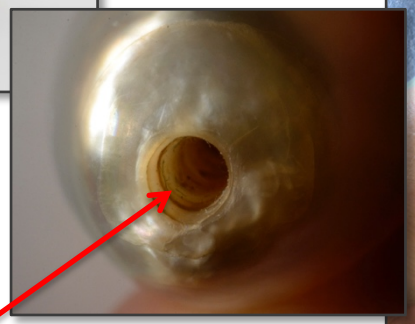


Figure: M.S. Krzemnicki, SSEF
Using background illustration
From www.fisheriesireland.ie

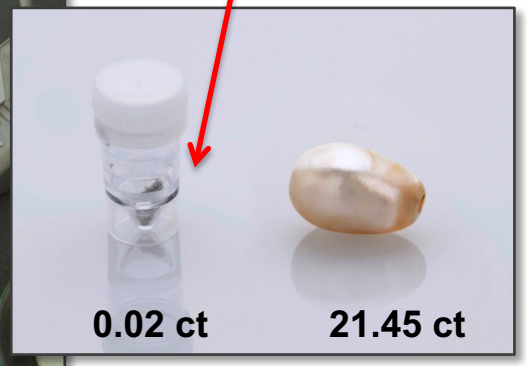
QUASI NON-DESTRUCTIVE SAMPLING (0.004 g)



A minute amount (less than 0.004 g) of calcium carbonate is taken from inside the drill-hole.

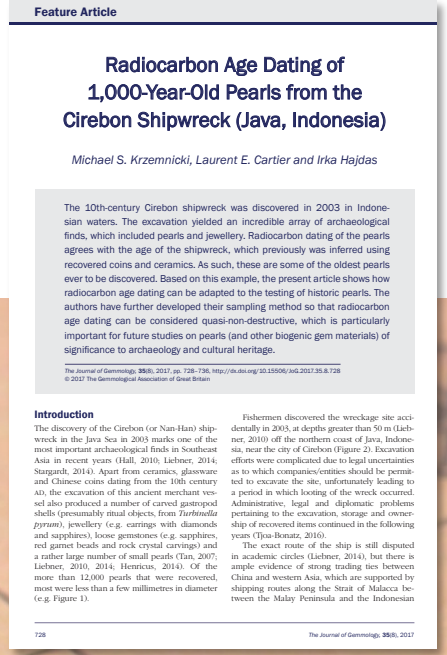
„quasi“ non-destructive testing even for objects of archaeological and cultural heritage.

0.02 ct powder of calcium carbonate for age dating.



PEARLS FROM CIREBON SHIPWRECK

1000 year old pearls
discovered in 2003 in the Java Sea (Indonesia).



Feature Article

Radiocarbon Age Dating of 1,000-Year-Old Pearls from the Cirebon Shipwreck (Java, Indonesia)

Michael S. Krzemnicki, Laurent E. Cartier and Irka Hajdas

The 10th-century Cirebon shipwreck was discovered in 2003 in Indonesian waters. The excavation yielded an incredible array of archaeological finds, which included pearls and jewellery. Radiocarbon dating of the pearls agrees with the age of the shipwreck, which previously was inferred using recovered coins and ceramics. As such, these are some of the oldest pearls ever to be discovered. Based on this example, the present article shows how radiocarbon age dating can be adapted to the testing of historic pearls. The authors have further developed their sampling method so that radiocarbon age dating can be considered quasi-non-destructive, which is particularly important for future studies on pearls (and other biogenic gem materials) of significance to archaeology and cultural heritage.

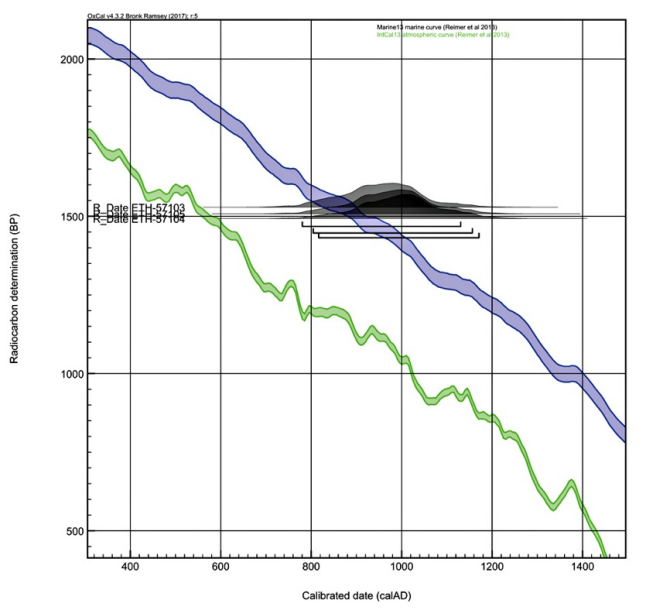
The Journal of Gemmology, **35**(8), 2017, pp. 728–736. http://dx.doi.org/10.15506/JoG.2017.35.8.728

Introduction

The discovery of the Cirebon (or Nan-Han) shipwreck in the Java Sea in 2003 marks one of the most important archaeological finds in Southeast Asia in recent years (Hall, 2010; Liebner, 2014; Stargardt, 2014). Apart from ceramics, glassware and Chinese coins dating from the 10th century AD, the excavation of this ancient merchant vessel also produced a number of carved gastropod shells (presumably ritual objects, from *Turbinella pyrum*), jewellery (e.g. earrings with diamonds and sapphires), loose gemstones (e.g. sapphires, red garnet beads and rock crystal carvings) and a rather large number of small pearls (Tan, 2007; Liebner, 2010, 2014; Henricus, 2014). Of the more than 12,000 pearls that were recovered, most were less than a few millimetres in diameter (e.g. Figure 1).

Fishermen discovered the wreckage site accidentally in 2003, at depths greater than 50 m (Liebner, 2010) off the northern coast of Java, Indonesia, near the city of Cirebon (Figure 2). Excavation efforts were complicated due to legal uncertainties as to which companies/entities should be permitted to excavate the site, unfortunately leading to a period in which looting of the wreck occurred. Administrative, legal and diplomatic problems pertaining to the excavation, storage and ownership of recovered items continued in the following years (Tjoa-Bonatz, 2016).

The exact route of the ship is still disputed in academic circles (Liebner, 2014), but there is ample evidence of strong trading ties between China and western Asia, which are supported by shipping routes along the Strait of Malacca between the Malay Peninsula and the Indonesian



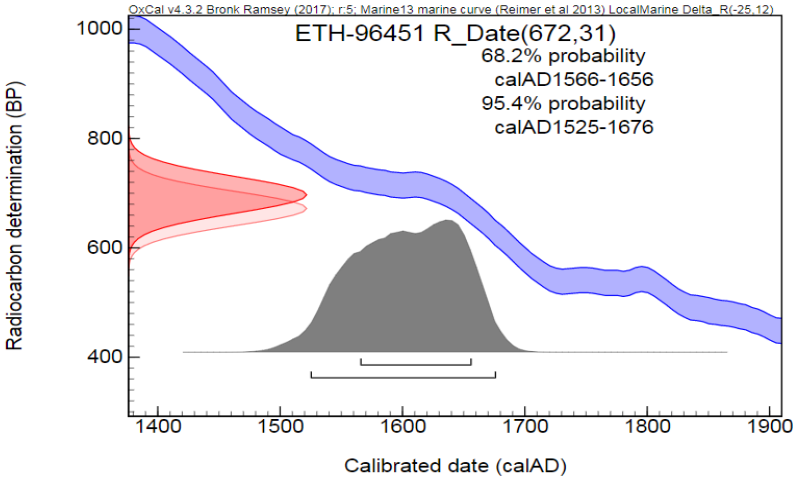
The ^{14}C ages perfectly fit with the archaeological dating of this shipwreck based on pottery and coins.

ANA MARIA PEARL

Documented since mid-19th century:

Originally belonging to Ana María de Sevilla (1828-1861). Probably fished during Hernán Cortéz's conquest of the Aztec Empire in the 16th century .

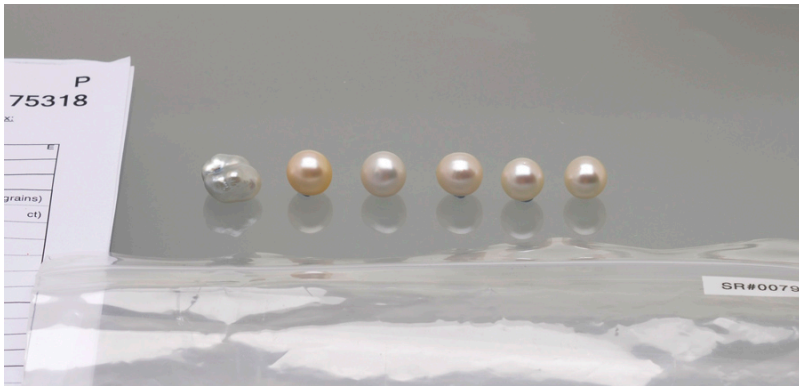
The radiocarbon analyses (carried out by ETH Zurich) date this pearl to the **16th – 17th century**, thus perfectly matching the documented historic provenance of this pearl.

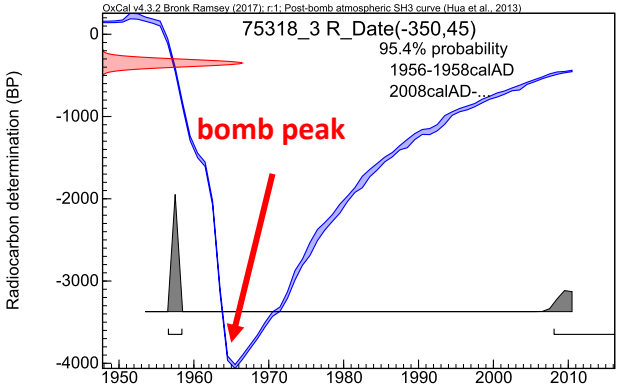


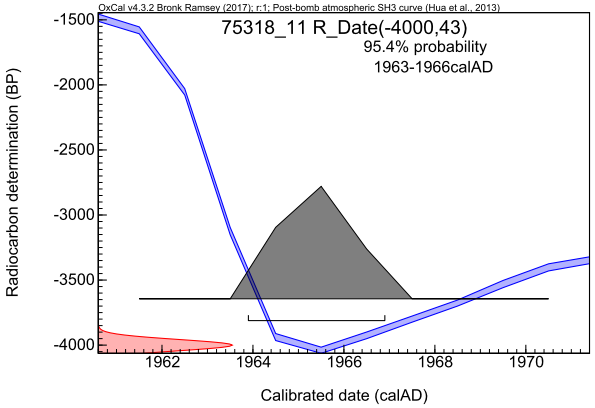
RECENT PEARL FORMATION

Cultured pearls formed around the “bomb” peak in 1966 (southern hemisphere). Early production from Kuri Bay, Australia‘s first pearl farm (first production 1956).

In the Paspaley pearl office in Darwin with Peter Bracher





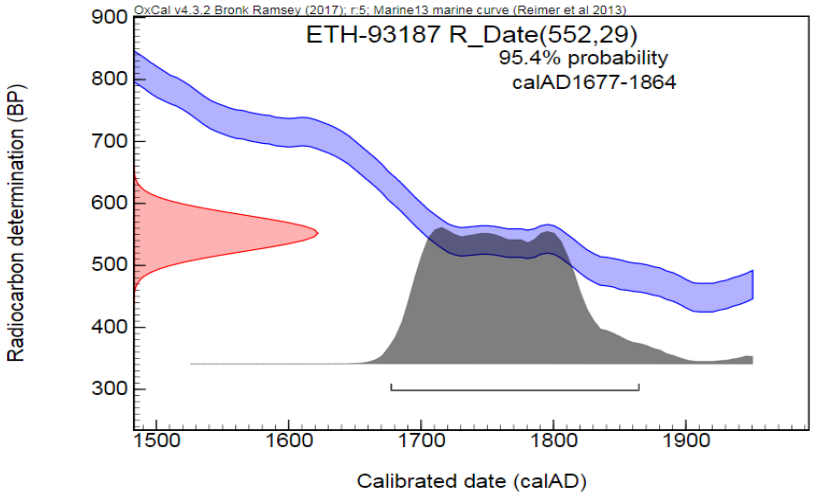


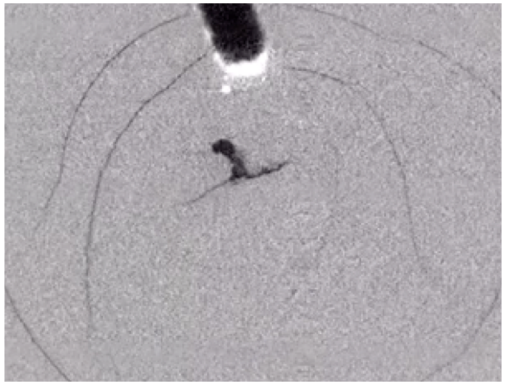
NP / CP SEPARATION

Supporting evidence due to radiocarbon age dating

This saltwater pearl shows internal structures which are also known in beadless cultured pearls (Krzemnicki et al. 2010, Sturman 2009).

The radiocarbon analyses (carried out by ETH Zurich) date this pearl to the **17th – 18th century**, distinctly pre-dating any pearl farming. The radiocarbon age thus supports the conclusion of natural pearl.





Micro X-ray tomographical section of pearl centre.

RADIOCARBON AGE DATING SERVICE

Since February 2017, the SSEF in collaboration with the Federal Institute of Technology (ETH Zurich) offers radiocarbon age dating of pearls as a service to our clients.

see: www.ssef.ch/press-releases/

AGE DATING OF NATURAL PEARLS AS A CLIENT SERVICE

The Swiss Gemmological Institute SSEF has become the first gem laboratory worldwide to introduce, as an additional service to clients, age dating of pearls using carbon-14 (14C). This scientific method can provide the pearl industry with new valuable information about the age of loose pearls and pearls in jewellery.

DOWNLOAD PDF

DNA FINGERPRINTING

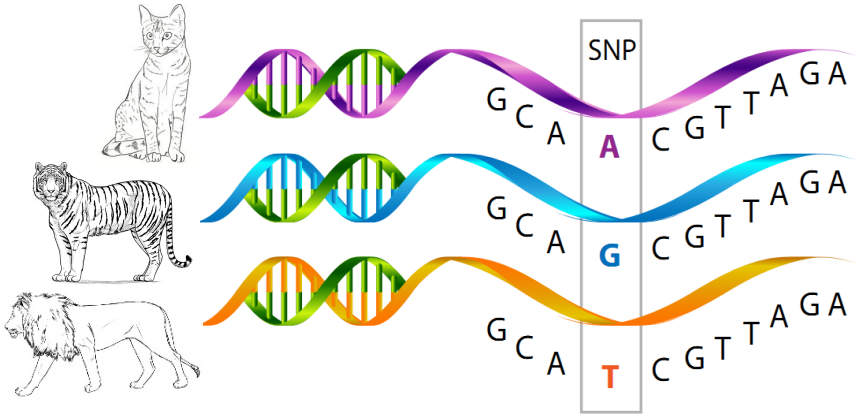
Species identification based on DNA fingerprinting.

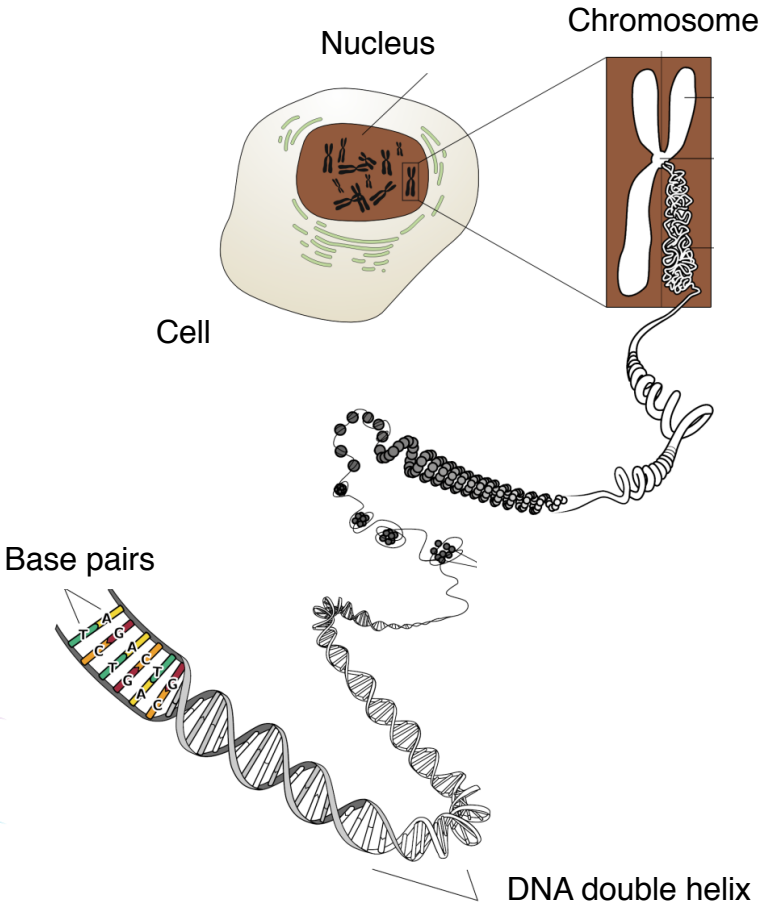
Apart from species identification, this method may assist in natural vs. cultured pearl separation, but may also provide information about the geographical “origin” of a pearl.

WHAT IS DNA?

- Deoxyribonucleic acid (DNA): Contains all the information an organism needs to develop, live and reproduce. It is formed by the four nucleobases (or 'bases') adenine (A), cytosine (C), guanine (G) and thymidine (T). The order of the bases (e.g. ATCGGTT…) codifies the specific instructions for any living organism.

- Genome: An organism's full set of DNA, including all of its genes.

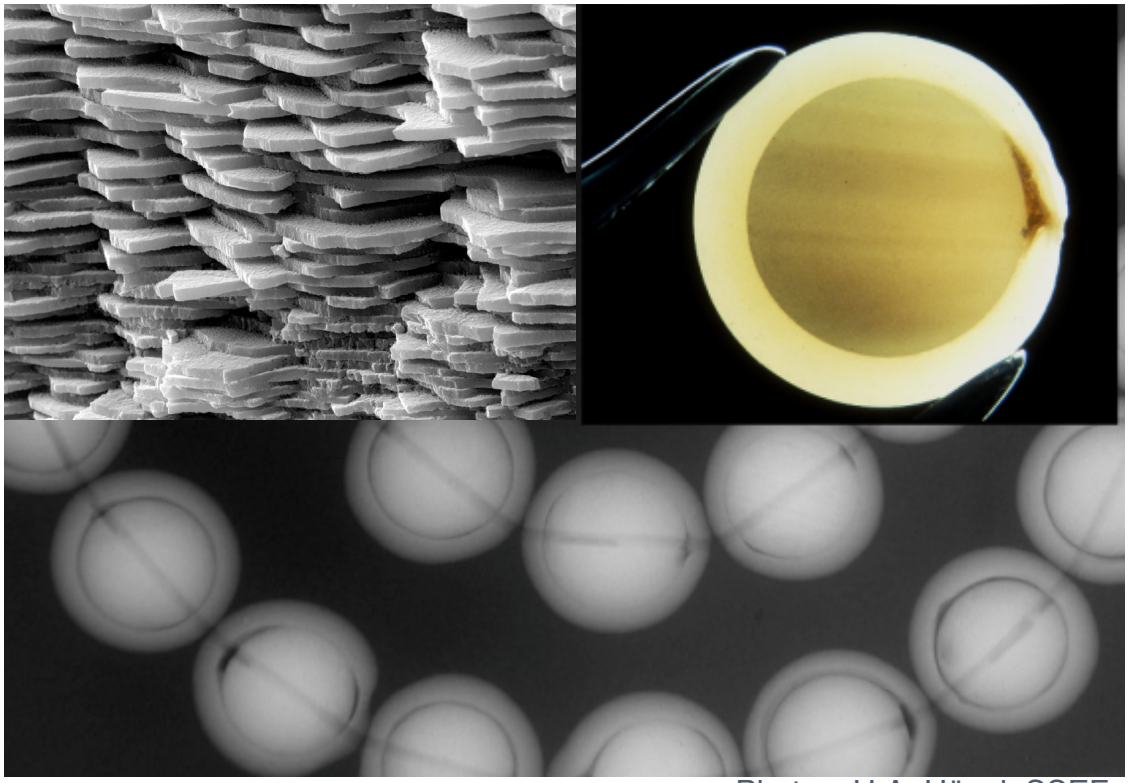




OYSTER DNA IN A PEARL?

The nacreous part of a pearl consists of approximately 92% $CaCO_3$, 4% organic matter (OM), 4% water and minute amounts of residual substances (Taylor & Strack, 2008).

The OM (consisting mostly of conchioline and porphyrines), which is also secreted by the pearl sac, serves as a framework for the $CaCO_3$ matrix during the biomineralization process. OM can also be found in concentrated pockets.



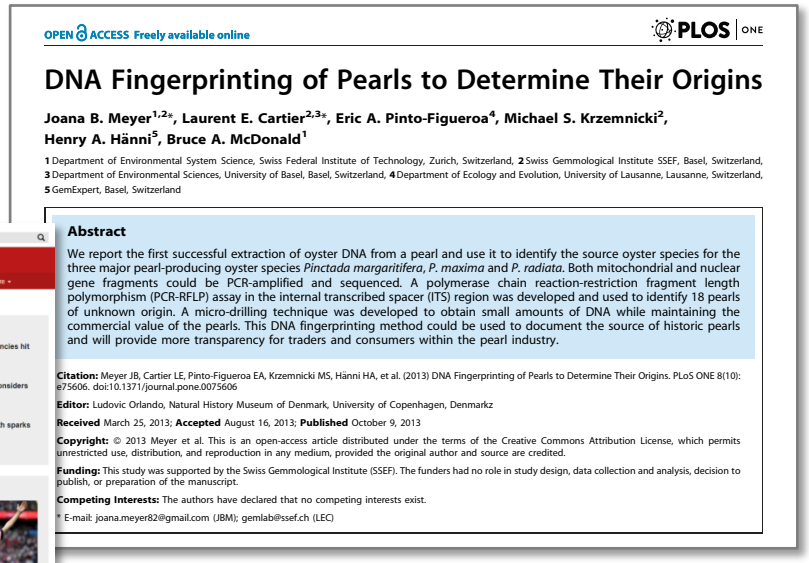
Photos: H.A. Hänni, SSEF

DNA EXTRACTION AND PREPARATION

Quasi non-destructive:

For recent coral DNA fingerprinting work we were able to extract DNA successfully with as low as **2.0 mg sample material**.

Methodology published in 2013 in PlosOne



OPEN ACCESS Freely available online

PLOS | ONE

DNA Fingerprinting of Pearls to Determine Their Origins

Joana B. Meyer[1,2]*, Laurent E. Cartier[2,3]*, Eric A. Pinto-Figueroa[4], Michael S. Krzemnicki[2], Henry A. Hänni[5], Bruce A. McDonald[1]

1 Department of Environmental System Science, Swiss Federal Institute of Technology, Zurich, Switzerland, **2** Swiss Gemmological Institute SSEF, Basel, Switzerland, **3** Department of Environmental Sciences, University of Basel, Basel, Switzerland, **4** Department of Ecology and Evolution, University of Lausanne, Lausanne, Switzerland, **5** GemExpert, Basel, Switzerland

Abstract

We report the first successful extraction of oyster DNA from a pearl and use it to identify the source oyster species for the three major pearl-producing oyster species *Pinctada margaritifera*, *P. maxima* and *P. radiata*. Both mitochondrial and nuclear gene fragments could be PCR-amplified and sequenced. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in the internal transcribed spacer (ITS) region was developed and used to identify 18 pearls of unknown origin. A micro-drilling technique was developed to obtain small amounts of DNA while maintaining the commercial value of the pearls. This DNA fingerprinting method could be used to document the source of historic pearls and will provide more transparency for traders and consumers within the pearl industry.

Citation: Meyer JB, Cartier LE, Pinto-Figueroa EA, Krzemnicki MS, Hänni HA, et al. (2013) DNA Fingerprinting of Pearls to Determine Their Origins. PLoS ONE 8(10): e75606. doi:10.1371/journal.pone.0075606

Editor: Ludovic Orlando, Natural History Museum of Denmark, University of Copenhagen, Denmarkz

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Competing Interests: The authors have declared that no competing interests exist.

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BBC NEWS

Science & Environment

Cutting edge science used to ID natural pearls

By Sylvia Smith
BBC News, Bahrain

20 May 2014

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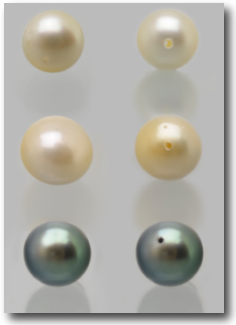
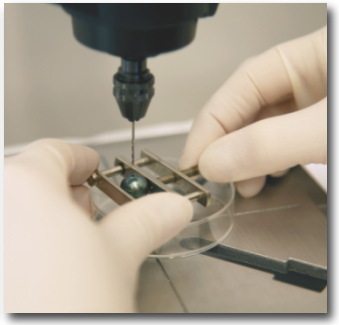
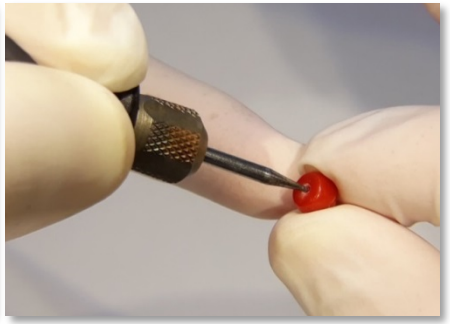
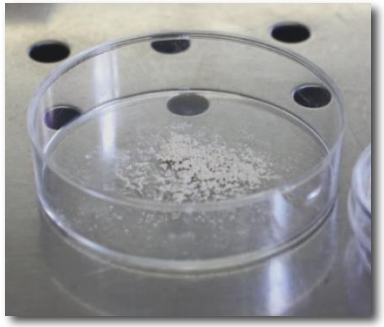
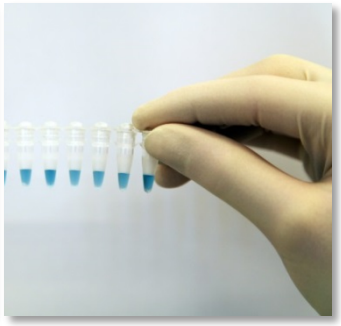
Megan Rapinoe's fight for equality





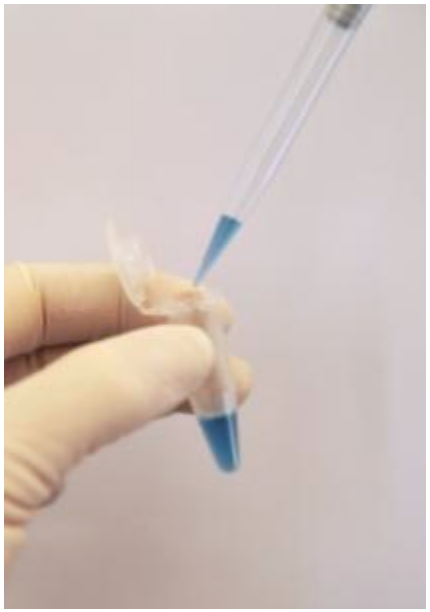


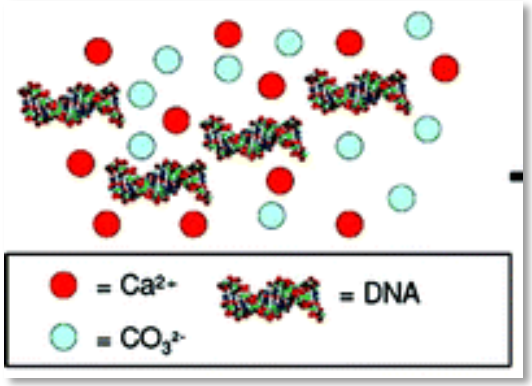




DNA EXTRACTION AND PREPARATION

- The negatively charged DNA molecule is known to have a high affinity for the Ca^{2+} ion of $CaCO_3$, which might enhance its conservation in pearls and corals.
- EDTA (0.5M pH=8, overnight 56°C) used to liberate DNA from $CaCO_3$ matrix.

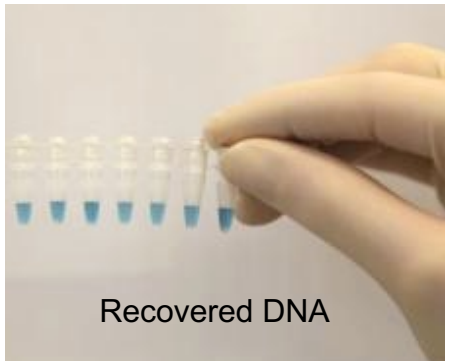


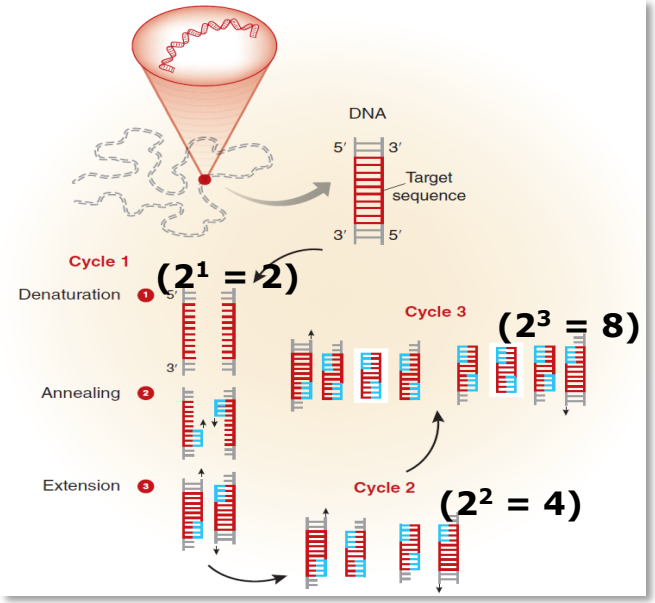


Source: Sommerdijk et al. (2007)

DNA CONCENTRATION AND AMPLIFICATION

- Kit needed to concentrate DNA from recovered sample powder.
- Having positive and negative controls is critical.
- Depending on the species to be identified, different markers in the genome are targeted (e.g. ITS2, 16S, Cox1, Cox2).
- DNA amplification involves using short DNA sequences (**primers**) to select the portion of a genome for amplification. In PCR, sample is repeatedly tempered to help a DNA replication enzyme synthesize the target DNA sequence.
- PCR can produce thousands to millions of copies of the target sequence, to be then analysed by visual inspection (e.g. gel electrophoresis) or by sequencing.

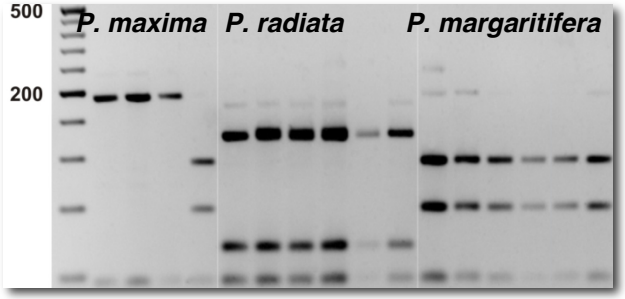


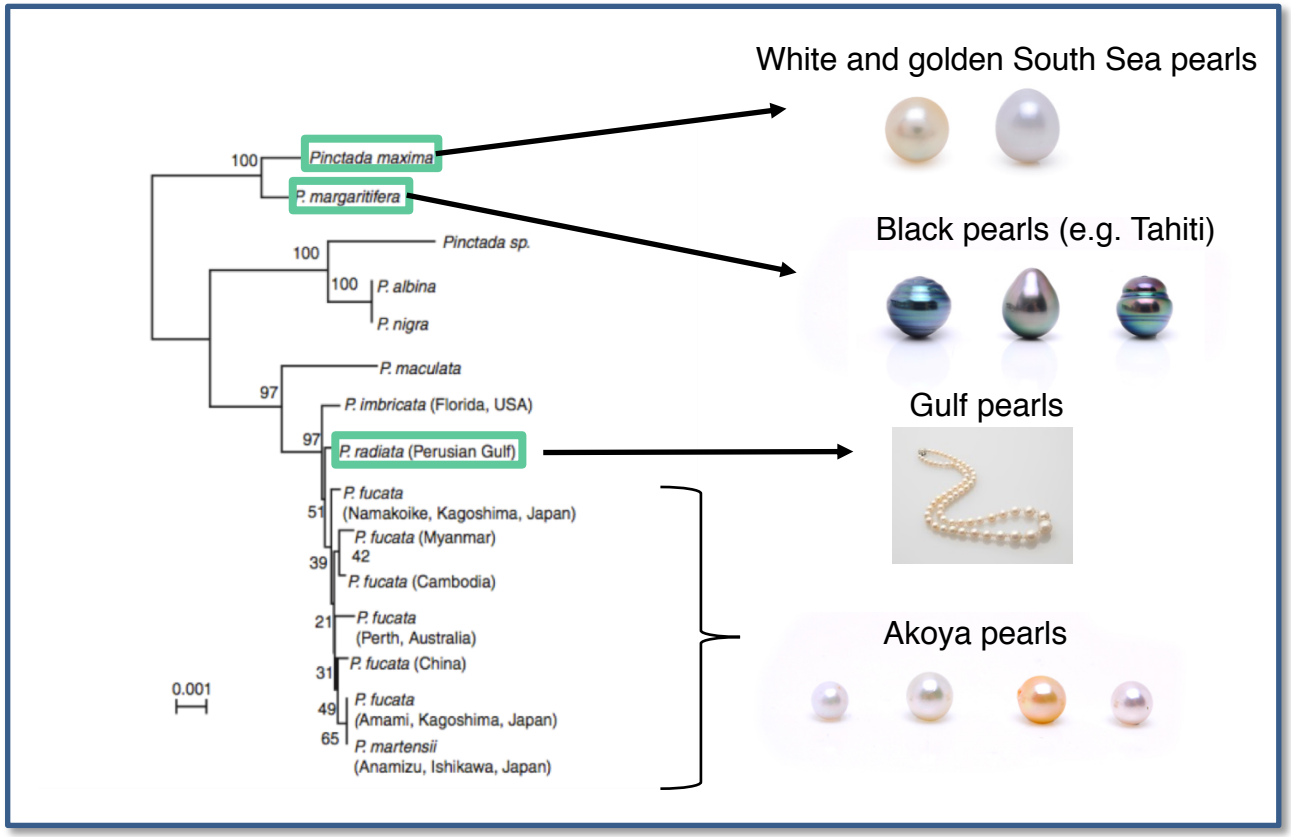


Source: Garibyan and Avashia, 2013

DNA SPECIES IDENTIFICATION OF PEARLS

- DNA species identification of pearls will be soon available as a service to our clients.

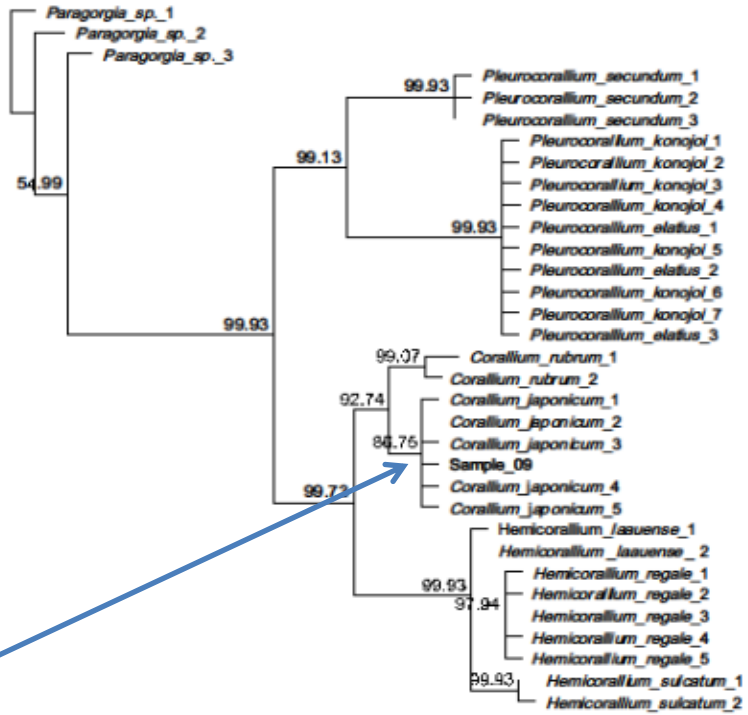




DNA SPECIES IDENTIFICATION OF CORALS

- DNA species identification of corals will be soon available as a service to our clients.
- SSEF offers this service in collaboration with the Institute of Forensic Medicine of the University Zurich, Switzerland

Sample CB009
from Corallium japonicum



DNA SPECIES IDENTIFICATION OF IVORY

- Ivory is any mammalian tooth or tusk of commercial interest.
- African (*Loxodonta africana*) and Asian elephants (*Elephas maximus*), along with their fossil relatives (e.g. *Mammuthus sp.*) belong to the mamallian order Proboscidea.

Published in JoG 2018

FEATURE ARTICLE

DNA Fingerprinting of Pearls, Corals and Ivory: A Brief Review of Applications in Gemmology

Laurent E. Cartier, Michael S. Krzemnicki, Bertalan Lendvay and Joana B. Meyer

Photo: Evgenia Arbugaeva, NatGeo

DNA SPECIES IDENTIFICATION OF IVORY

Since September 2019, the SSEF in collaboration with the Institute of Forensic Medicine (University Zurich) offers DNA species identification of ivory as a service to our clients.

see: www.ssef.ch/press-releases/

SSEF INTRODUCES DNA SPECIES IDENTIFICATION OF IVORY AS CLIENT SERVICE

SSEF launches a services that provides a scientific method to identify the species of ivory being used in jewellery and ornamental objects, in order to determine whether it is CITES-listed elephant ivory or non-listed mammoth ivory.

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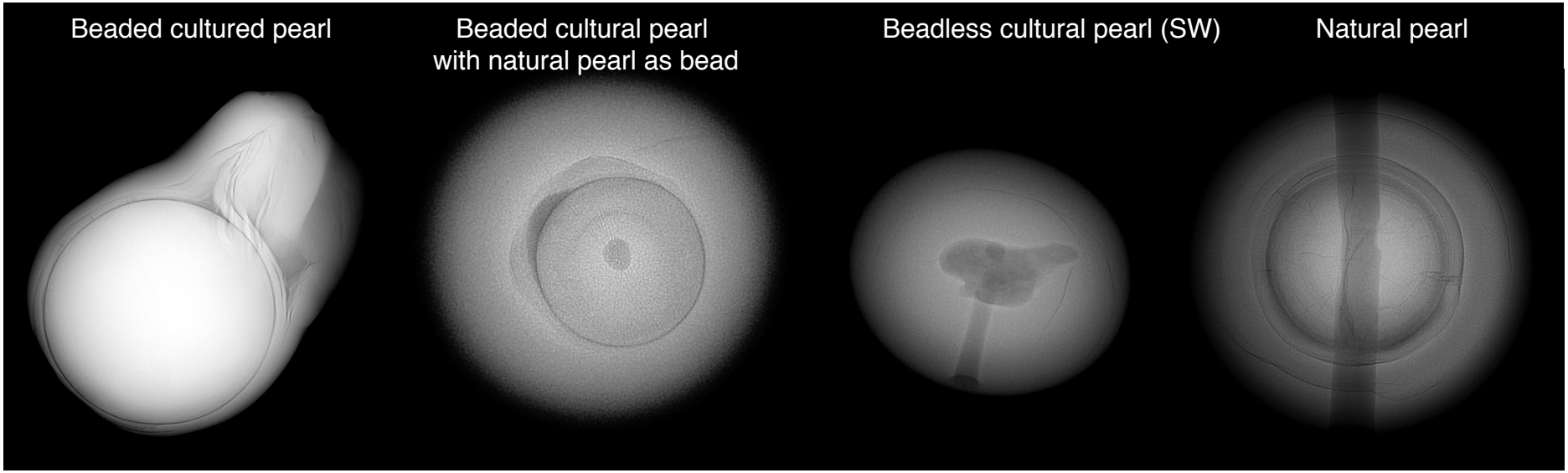
NOVEL RADIOGRAPHIC METHODS

- **X-ray phase contrast and X-ray darkfield (scattering)**
- **Neutron radiography and tomography**

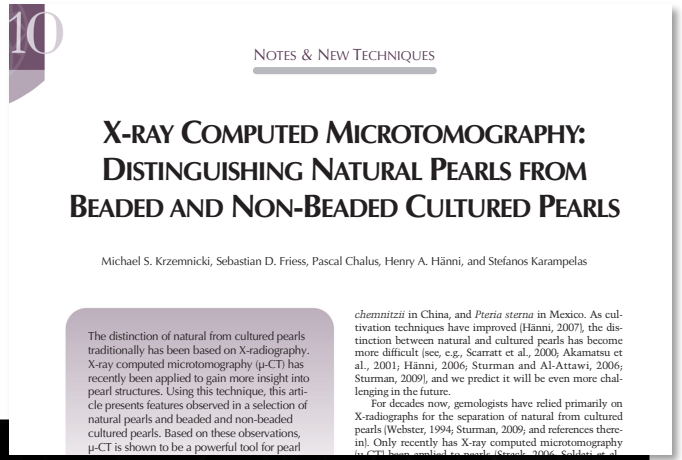
Both approaches visualise internal structures in pearls and may be used complementary to classic X-ray radiography (attenuation).

CLASSIC RADIOGRAPHY

Since decades, X-ray radiography (attenuation) is the most important method to visualise internal structures in pearls.



X-RAY MICROTOMOGRAPHY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NOTES & NEW TECHNIQUES

X-RAY COMPUTED MICROTOMOGRAPHY: DISTINGUISHING NATURAL PEARLS FROM BEADED AND NON-BEADED CULTURED PEARLS

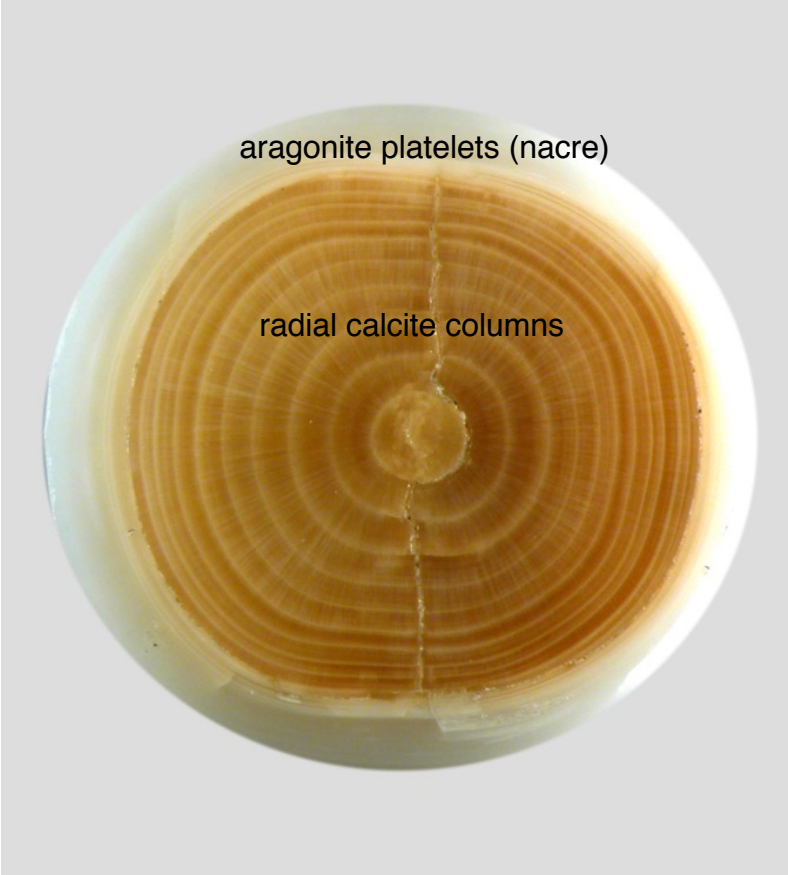
Michael S. Krzemnicki, Sebastian D. Friess, Pascal Chalus, Henry A. Hänni, and Stefanos Karampelas

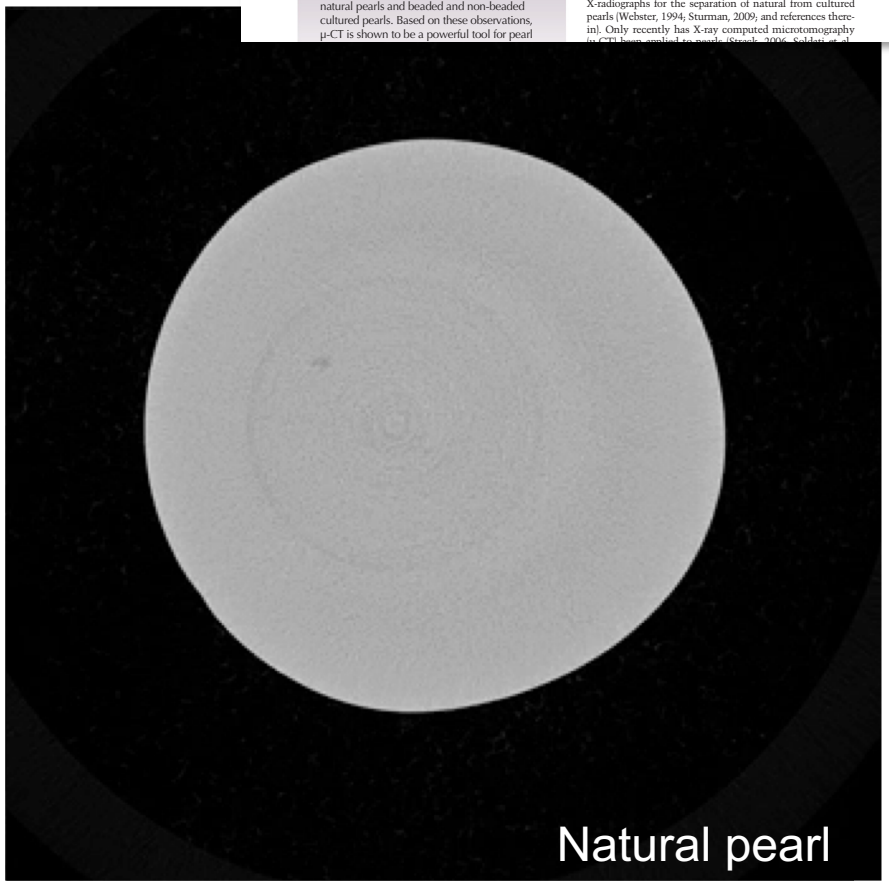
The distinction of natural from cultured pearls traditionally has been based on X-radiography. X-ray computed microtomography (μ-CT) has recently been applied to gain more insight into pearl structures. Using this technique, this article presents features observed in a selection of natural pearls and beaded and non-beaded cultured pearls. Based on these observations, μ-CT is shown to be a powerful tool for pearl

chemnitzii in China, and *Pteria sterna* in Mexico. As cultivation techniques have improved (Hänni, 2007), the distinction between natural and cultured pearls has become more difficult (see, e.g., Scarratt et al., 2000; Akamatsu et al., 2001; Hänni, 2006; Sturman and Al-Attawi, 2006; Sturman, 2009), and we predict it will be even more challenging in the future.

For decades now, gemologists have relied primarily on X-radiographs for the separation of natural from cultured pearls (Webster, 1994; Sturman, 2009; and references therein). Only recently has X-ray computed microtomography

X-ray microtomography allows 3D visualisation of internal structures in pearls.

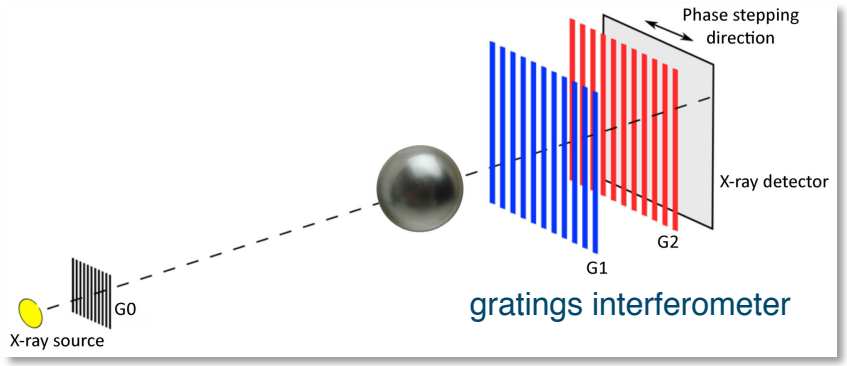




X-RAY PHASE CONTRAST &DARKFIELD IMAGING

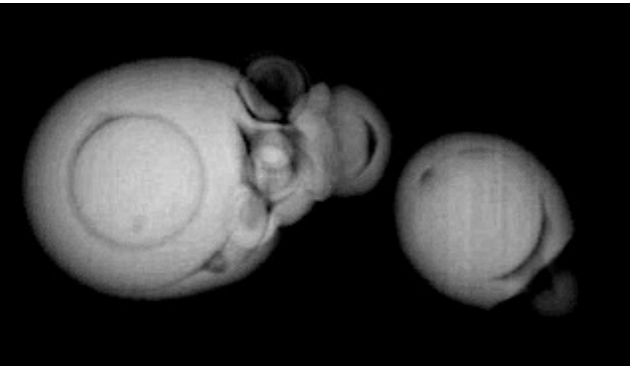
By using two moving gratings, it is possible to get simultaneously three X-ray images of a pearl:

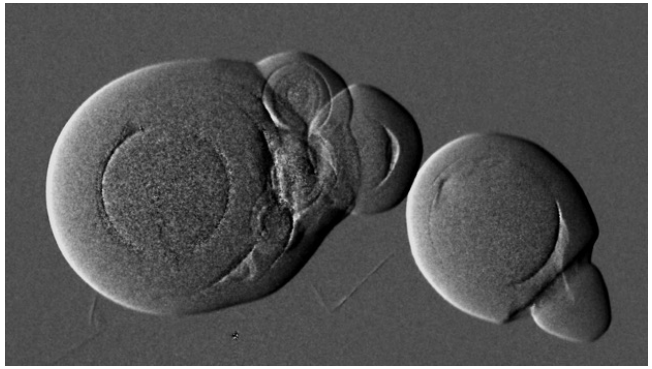
- absorption (classic radiography)
- refraction (phase contrast)
- and scattering (darkfield)

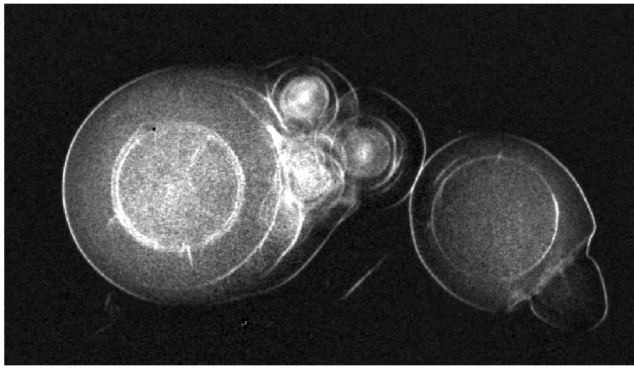


X-ray setup with gratings interferometer

Absorption	Refraction	Scattering



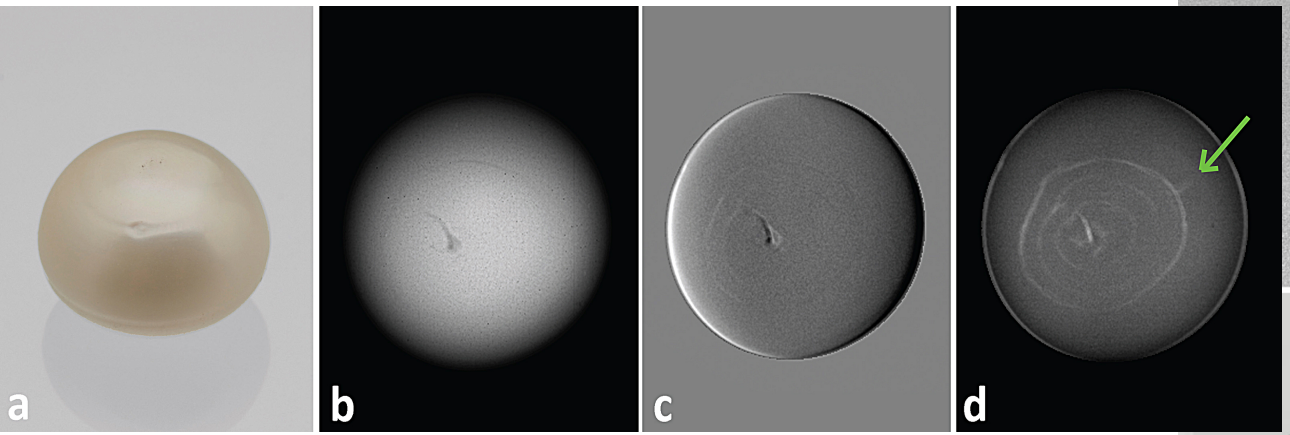


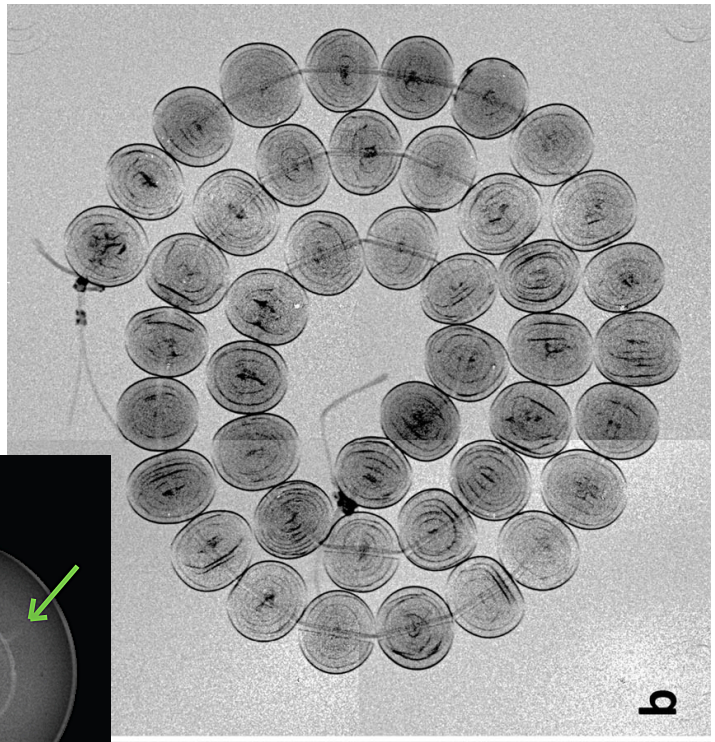


Beaded cultured pearl (SW) in radiography, phase contrast, and darkfield imaging

X-RAY PHASE CONTRAST &DARKFIELD IMAGING

Structures which are difficult to see in classic radiography can be observed in phase contrast and darkfield imaging with more detail

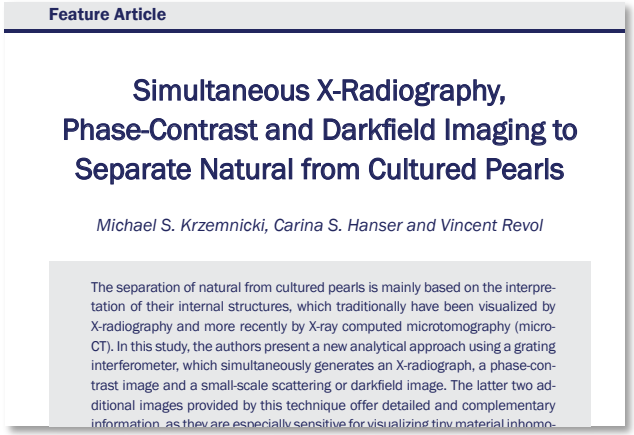




Above: beadless cultured pearl (FW) in radiography, phase contrast, and darkfield imaging.

Right: necklace of beadless cultured pearls (FW) from China in darkfield imaging (inversed) .

X-RAY PHASE CONTRAST & DARKFIELD IMAGING

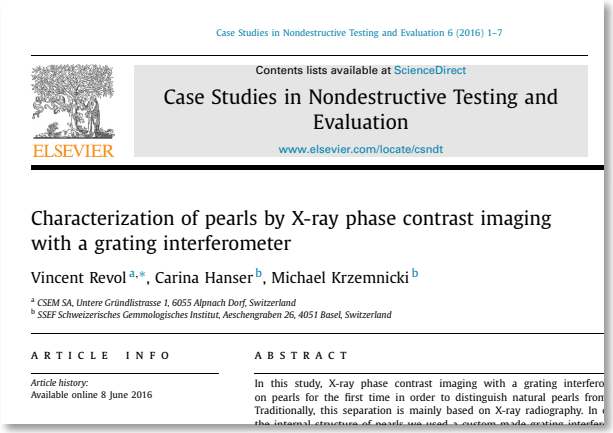


Feature Article

Simultaneous X-Radiography, Phase-Contrast and Darkfield Imaging to Separate Natural from Cultured Pearls

Michael S. Krzemnicki, Carina S. Hanser and Vincent Revol

The separation of natural from cultured pearls is mainly based on the interpretation of their internal structures, which traditionally have been visualized by X-radiography and more recently by X-ray computed microtomography (micro-CT). In this study, the authors present a new analytical approach using a grating interferometer, which simultaneously generates an X-radiograph, a phase-contrast image and a small-scale scattering or darkfield image. The latter two additional images provided by this technique offer detailed and complementary information, as they are especially sensitive for visualizing tiny material inhomo-



Case Studies in Nondestructive Testing and Evaluation 6 (2016) 1–7

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Characterization of pearls by X-ray phase contrast imaging with a grating interferometer

Vincent Revol [a,*], Carina Hanser [b], Michael Krzemnicki [b]

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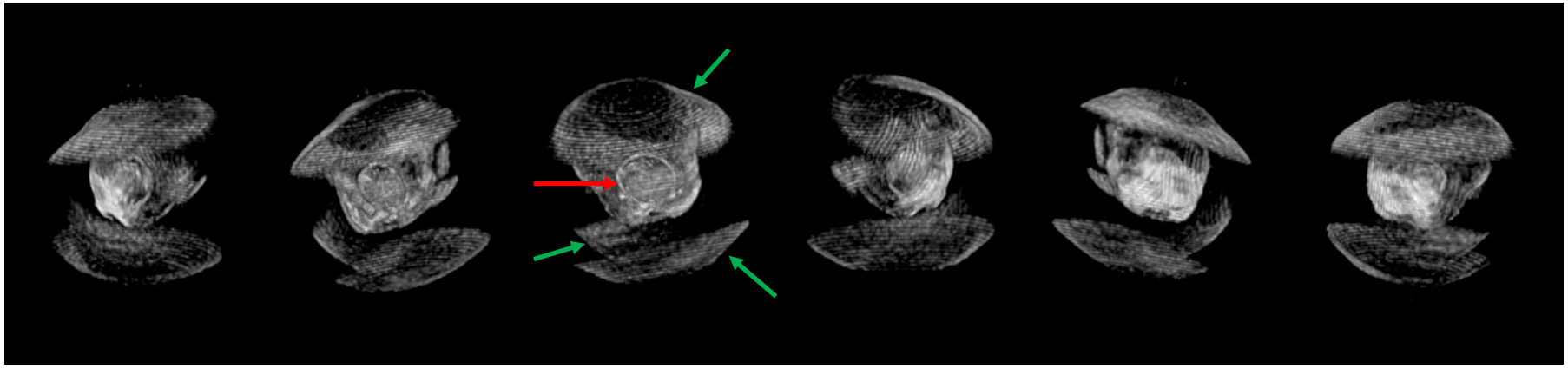
ABSTRACT

In this study, X-ray phase contrast imaging with a grating interfero
on pearls for the first time in order to distinguish natural pearls from
Traditionally, this separation is mainly based on X-ray radiography. In

It is even possible to do X-ray tomography in phase contrast or darkfield modes.



3D rendering of natural pearl in darkfield (scattering) mode.
red arrow: organic rich spherical core, green arrows: onion-like growth structures in nacre.

NEUTRON IMAGING

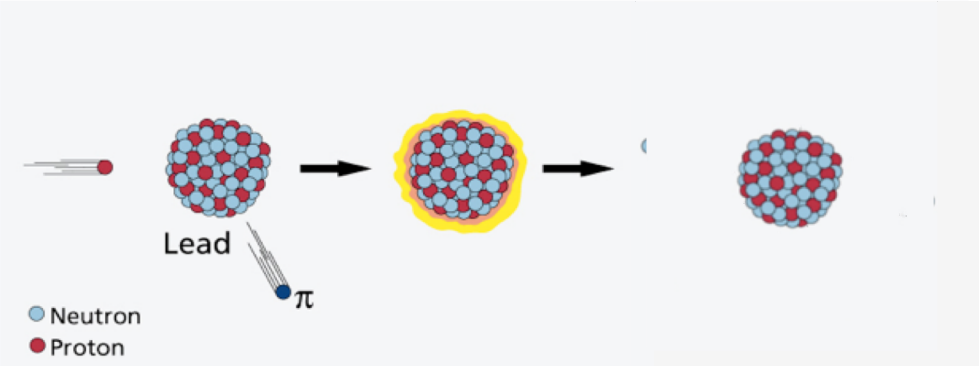
Research in collaboration with the Laboratory for Neutron Scattering and Imaging, **Paul Scherrer Institute PSI (Synchrotron & Neutron Spallation Source)**, Villingen, Switzerland

NEUTRON IMAGING

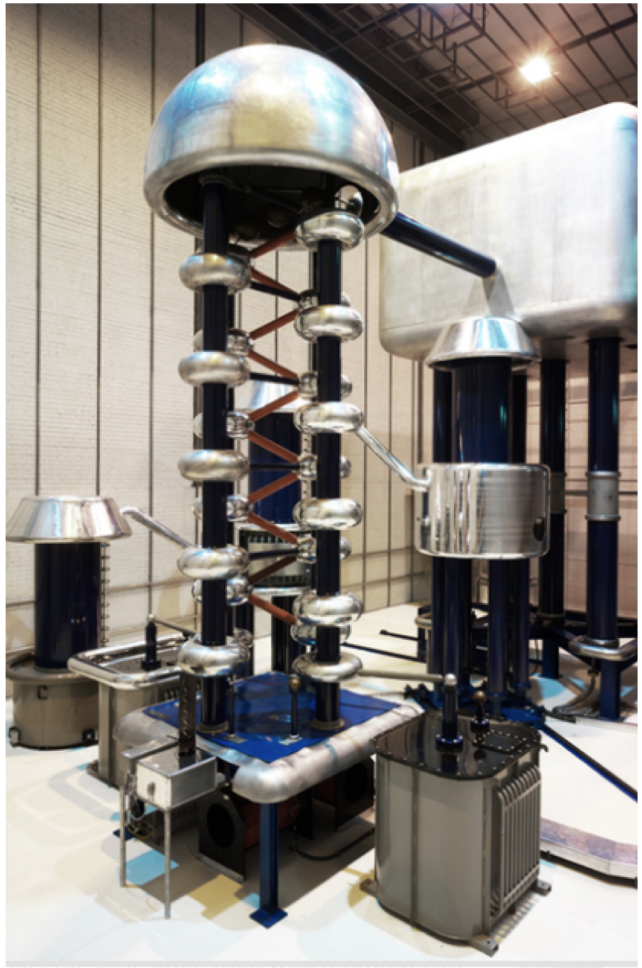
The neutron beam for imaging is produced at **SINQ spallation source** (PSI Switzerland).

- PSI proton accelerator produces beam of fast protons (~ 80 % of the speed of light).

- Proton beam strikes a block of lead (**target**). If a fast proton collides with a lead nucleus, the nucleus will be heated up and ejects 10 to 20 neutrons.



- These neutrons are slowed down by collisions with the nuclei of heavy water (deuterium) to be used as neutron beam for imaging analyses.

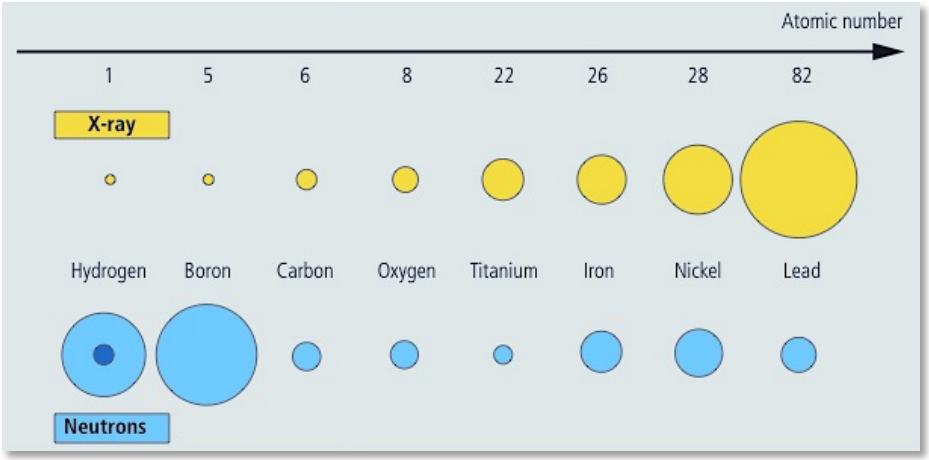


The PSI Cockroft-Walton accelerator – the first stage of the proton accelerator facility

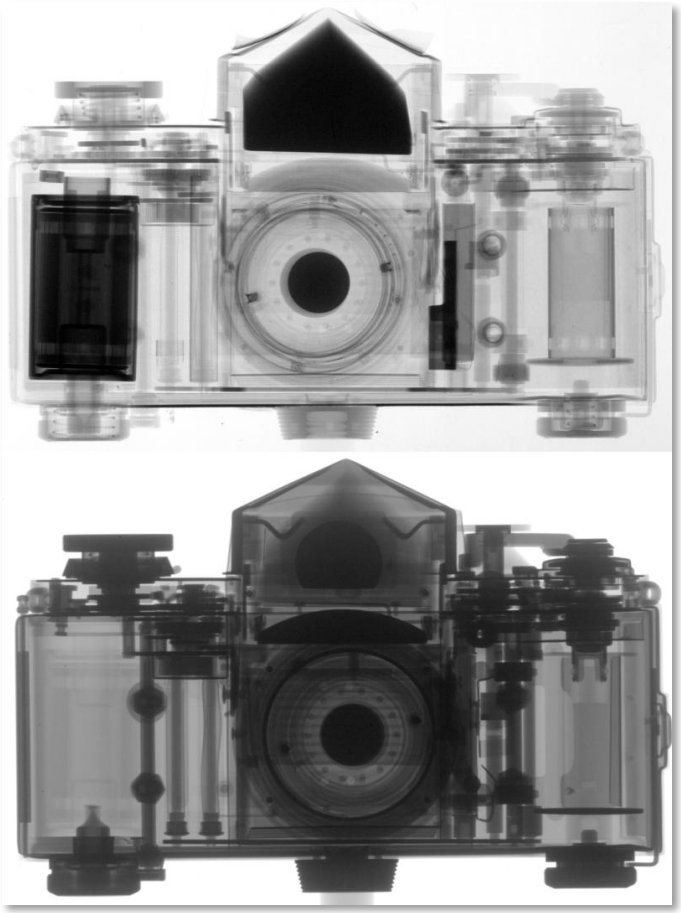
Photos and Scheme: Paul Scherrer Institute PSI

NEUTRON IMAGING

While X-rays are attenuated more effectively by heavier materials like metals, neutrons make it possible to image some light materials such as hydrogenous substances with high contrast:



Interaction behaviour of different atoms with X-rays and neutrons. The larger the diameter, the stronger the interaction (absorption).

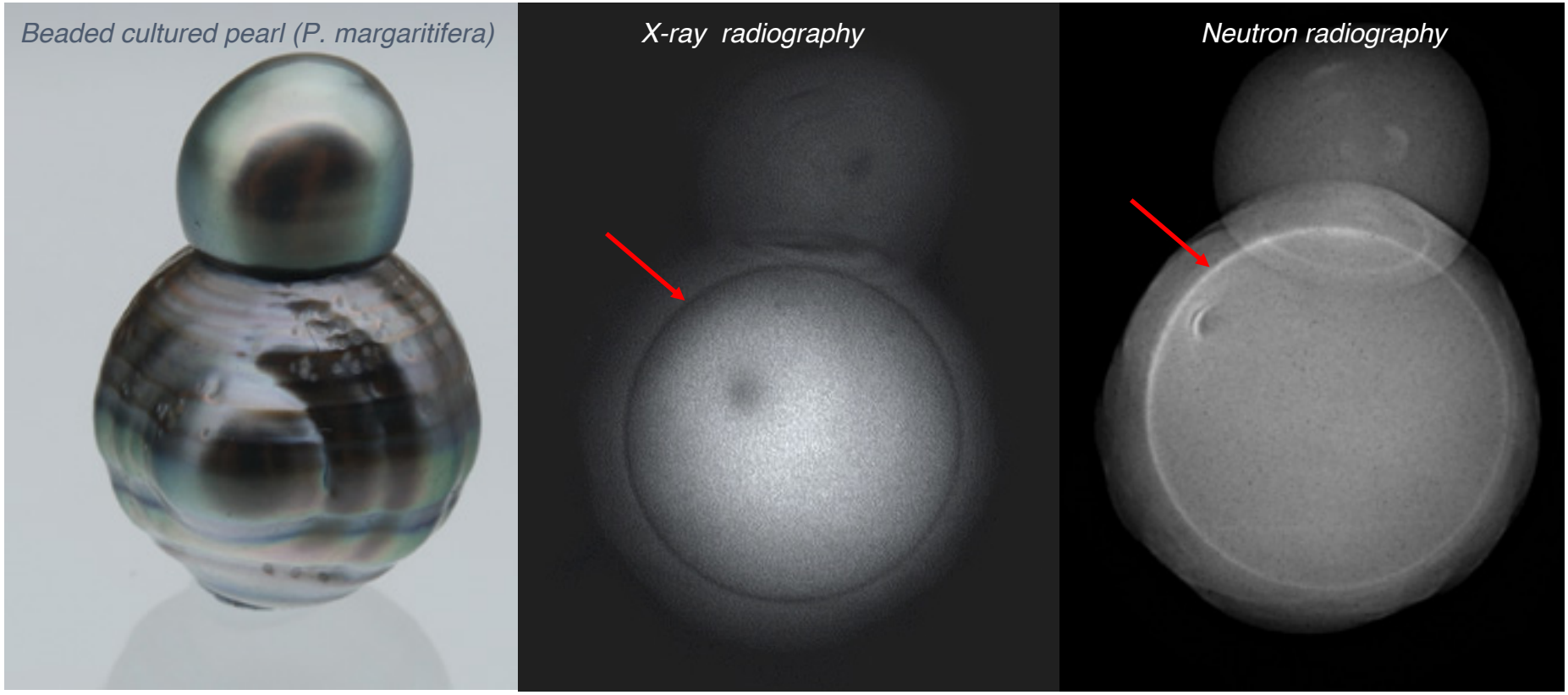


Radiograph of a camera by neutrons (top) showing details of the plastic parts and by X-rays (bottom) revealing the metal parts of the camera.

Photos and Scheme: Paul Scherrer Institute PSI

NEUTRON IMAGING

Neutron images are best analysed by direct comparison with X-ray images.

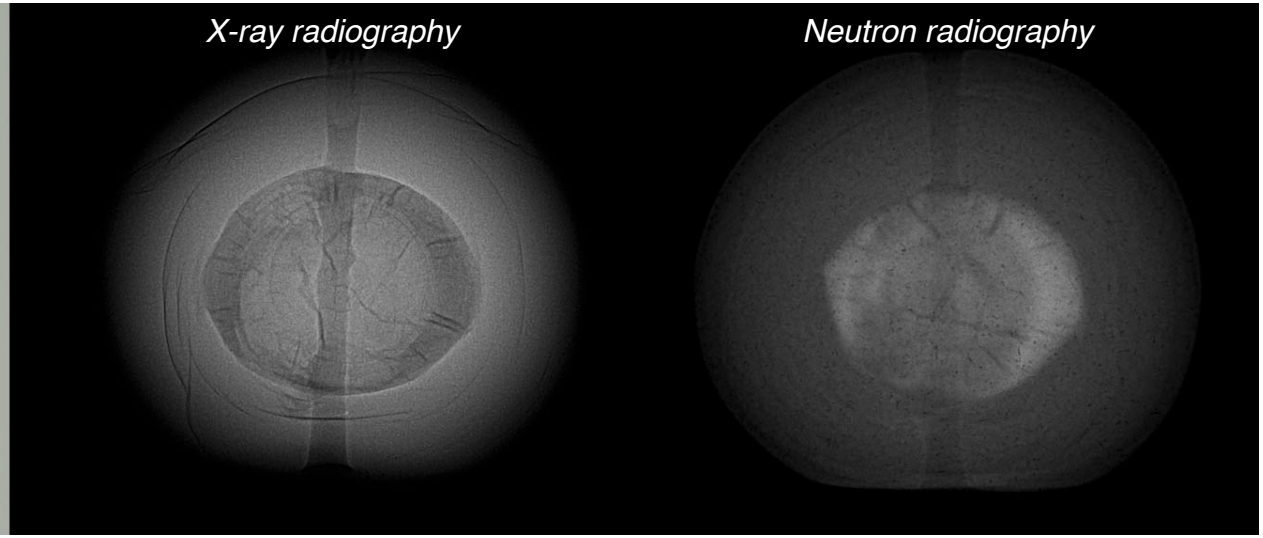


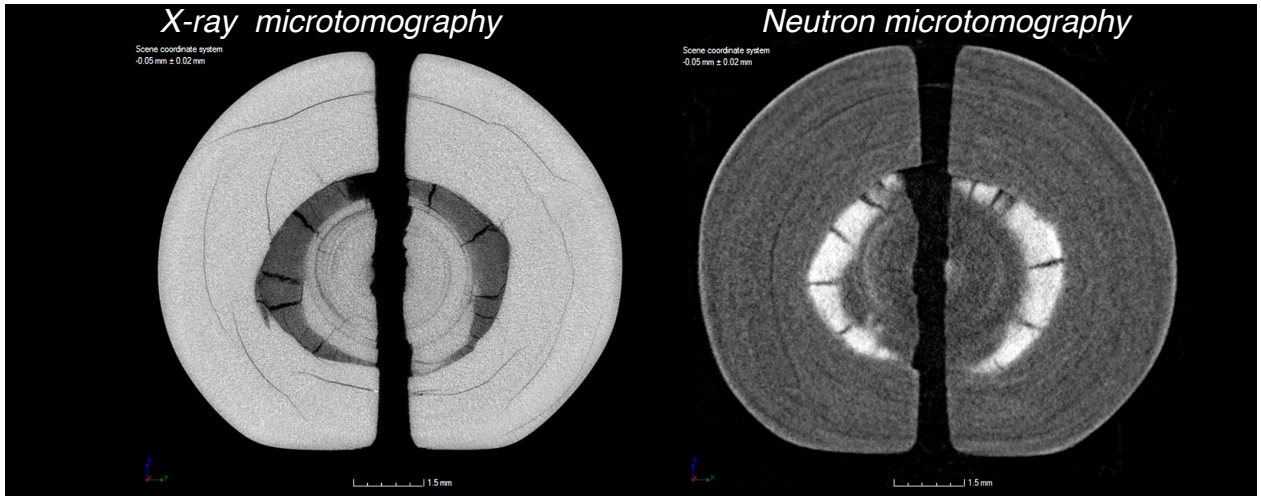
Beaded cultured pearl in X-ray radiography and neutron radiography, revealing the inverse absorption behaviour of these two emission sources with organic matter (hydrogen-enriched) in pearls
red arrows: organic layer (conchioline) wrapping the inserted bead in this cultured pearl.

NEUTRON IMAGING

Similar to X-rays, it is also possible to use neutrons for microtomography.

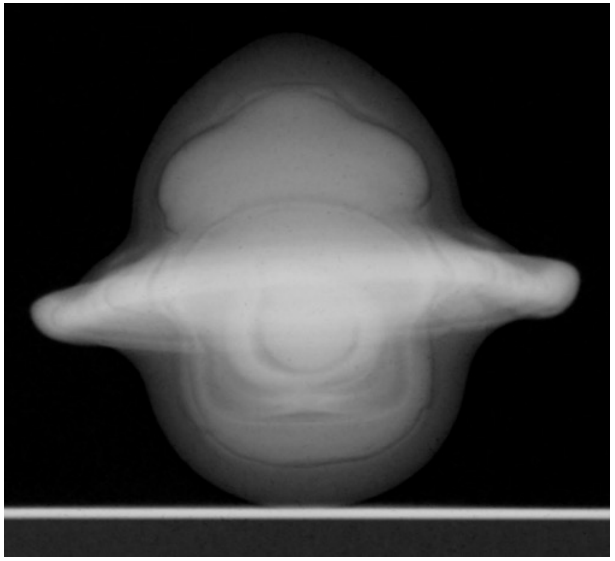
Natural pearl (Pinctada radiata)





NEUTRON IMAGING

Natural pearl (Pinctada maxima) from NW Australia (Paspaley)



FEATURE ARTICLE

Neutron Radiography and Tomography: A New Approach to Visualize the Internal Structures of Pearls

Carina S. Hanser, Michael S. Krzemnicki, Christian Grünzweig, Ralph P. Harti, Benedikt Betz and David Mannes

Non-destructive imaging of the internal structures of pearls has so far been mainly based on X-ray imaging methods. As organic matter is almost transparent to X-rays, the identification of some structures can be difficult. This study shows that neutron imaging can be a helpful complementary method to visualize structures inside pearls beyond standard X-ray radiography and tomography, as neutrons are highly attenuated by hydrogen-bearing (organic) matter within pearls. The use of neutron radiography and tomography is shown for selected natural and cultured pearls (beaded and non-beaded). In addition, we present neutron phase contrast and darkfield images of a beaded cultured pearl, in analogy to X-ray phase contrast and darkfield imaging described in a previous study. While neutron imaging of pearls is particularly useful for understanding material inhomogeneities and void structures, this methodology is currently only available at large-scale facilities that are equipped to deal with nuclear reactions.

The Journal of Gemmology, **36**(1), 2018, pp. 54–63, http://dx.doi.org/10.15506/JoG.2018.36.1.54

INTRODUCTION

For about a century, the identification of pearls (i.e. differentiating between their natural or cultured formation; Figure 1) has been a crucial task for gemmologists, given the enormous differences in their rarity and price. Pearl identification is mostly based on the interpretation of internal structures, traditionally visualized by imaging methods such as X-ray radiography (Anderson, 1932; Scarratt et al., 2000; Strack, 2006; Sturman, 2009) and, more recently, X-ray computed microtomography (Wehrmeister et al., 2008; Karampelas et al., 2010; Krzemnicki et al., 2010; Otter et al., 2014) and X-ray phase contrast and darkfield imaging (Revol et al., 2016; Krzemnicki et al., 2017).

With this article, the authors present neutron imaging as a new and complementary method to visualize internal pearl structures. This paper is part of a larger joint research study between author CSH, the SSEF Swiss Gemmological Institute and the Paul Scherrer Institute, in which a total of 32 samples (13 natural and 19 cultured pearls) were investigated with the aim of comparing various imaging methods for pearl testing (Hanser, 2015). It is a follow-up to the article about X-ray phase contrast and darkfield imaging that was recently published in *The Journal* (Krzemnicki et al., 2017).

Similar to X-rays, neutrons can be applied for imaging purposes because of their selective attenuation via absorption and scattering by certain materials. But unlike X-rays, neutrons interact with the atomic nuclei only. Thus, even some lighter elements such as hydrogen strongly attenuate neutrons, whereas X-rays (which interact with the electrons of atoms) are very weakly attenuated by light elements and are increasingly affected by heavier elements (directly related to the element's atomic number). Generally speaking, neutron radiography images are inverse to

CONCLUSIONS

- Today, new scientific methods are available for pearl testing, these include:

  - **Radiocarbon age dating** using 2-3 mg of nacre ("quasi non-destructive") as client service.
  - **DNA species identification** using 2-3 mg of material ("quasi non-destructive") as client service.
  - **Novel imaging methods**, such as X-ray phase contrast and darkfield radiography and tomography and neutron radiography and tomography.

- However, conclusions of pearl testing still are (and will be in future) expert opinions based on the educated interpretation of observations and data.

- Scientific research on pearls, their formation, and their internal structure are fascinating topics which require a multi-disciplinary approach involving mineralogy (biomineralisation), biology, forensics, and the latest imaging technology.

THANK YOU FOR YOUR ATTENTION

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