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TOOLS FOR IDENTIFICATION: FORENSIC RADIOLOGY AND NEW DEVELOPMENTS IN DNA SAMPLE TYPES FOR DECOMPOSED AND BURNT HUMAN REMAINS

Prof. Noel Woodford / Director

ICRC Workshop: Management and Identification of Decedent Migrants

No Conflicts of Interest to declare

The following presentation contains images of deceased persons

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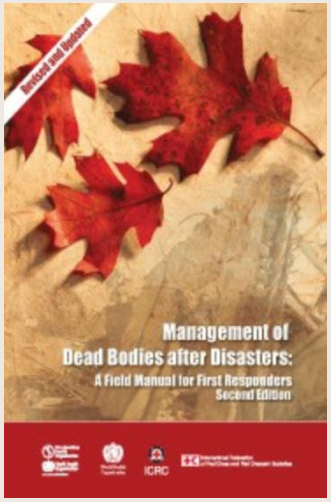
TO BE DISCUSSED

Identification Phases

1. Scene
2. **Mortuary/laboratory**
   - Radiology/Molecular
3. **Ante-mortem**
   - Information/reference sample
4. Reconciliation- contemporaneous or deferred
5. Debriefing



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TYPES OF REMAINS

Preserved-intact

Decomposed

Fire-affected

Fragmented

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VICTORIA 2009 AND PNG 2011
LESSONS LEARNED AND APPLIED

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IDENTIFICATION

Primary

- Dental
- DNA
- Fingerprints

- Medical implants

IDENTIFICATION METHODS

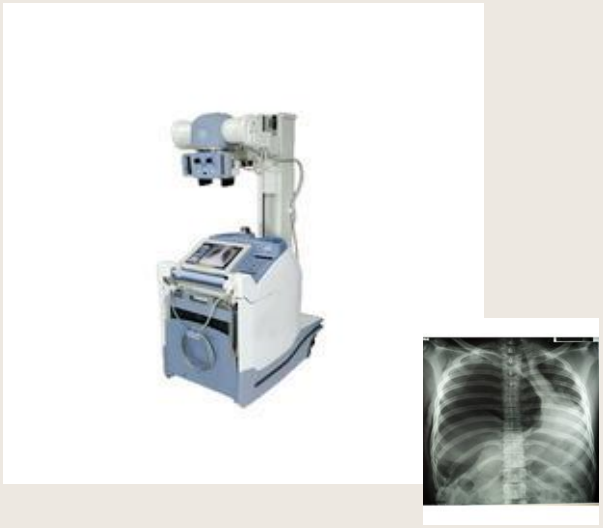
Secondary/supportive

- Visual
- Clothing
- Documents
- Jewellery
- Circumstances
- Scars, tattoos, deformities
- Others- modelling, superimposition

RADIOLOGY MODALITIES

- Plain X-ray
- Image intensifier

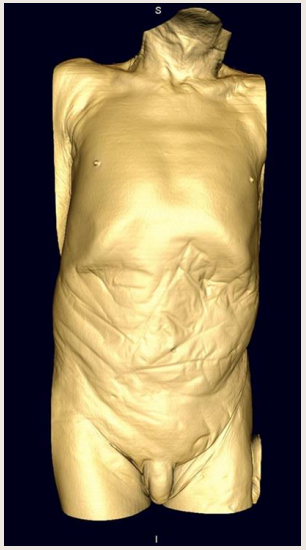
- CT
- (MRI)



CT ADVANTAGES

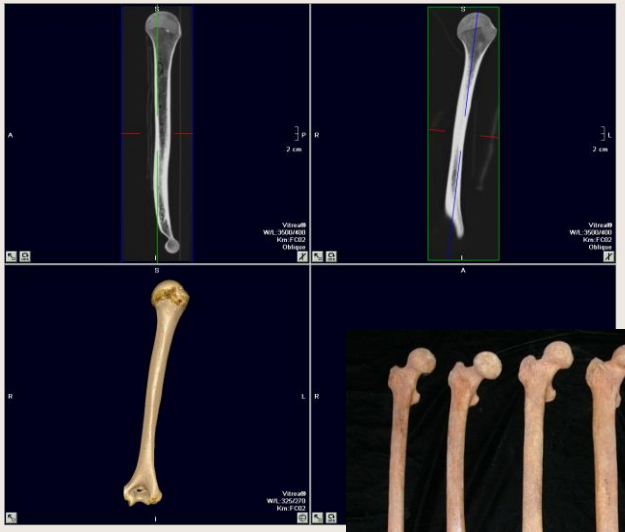
- **Digital permanent record**
- **Remains in body bag**
  - minimise hazard risk
  - minimise evidence loss
- **Data for deferred/remote pathological, odontological, anthropological examination**
  - Reconciliation/re-allocation

CT- ANALYSIS



Scan once, post-process many times

- **1º survey- initial radiological CT report**
- **2º survey- specific dental, anthropological assessment**
- **3º survey- retrospective radiological review**



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CT REPORTING

Sex

Age

Natural Disease

Specific identifiers

- **Dentition**
- **Surgical implants**

- **ISFRI-DVI***

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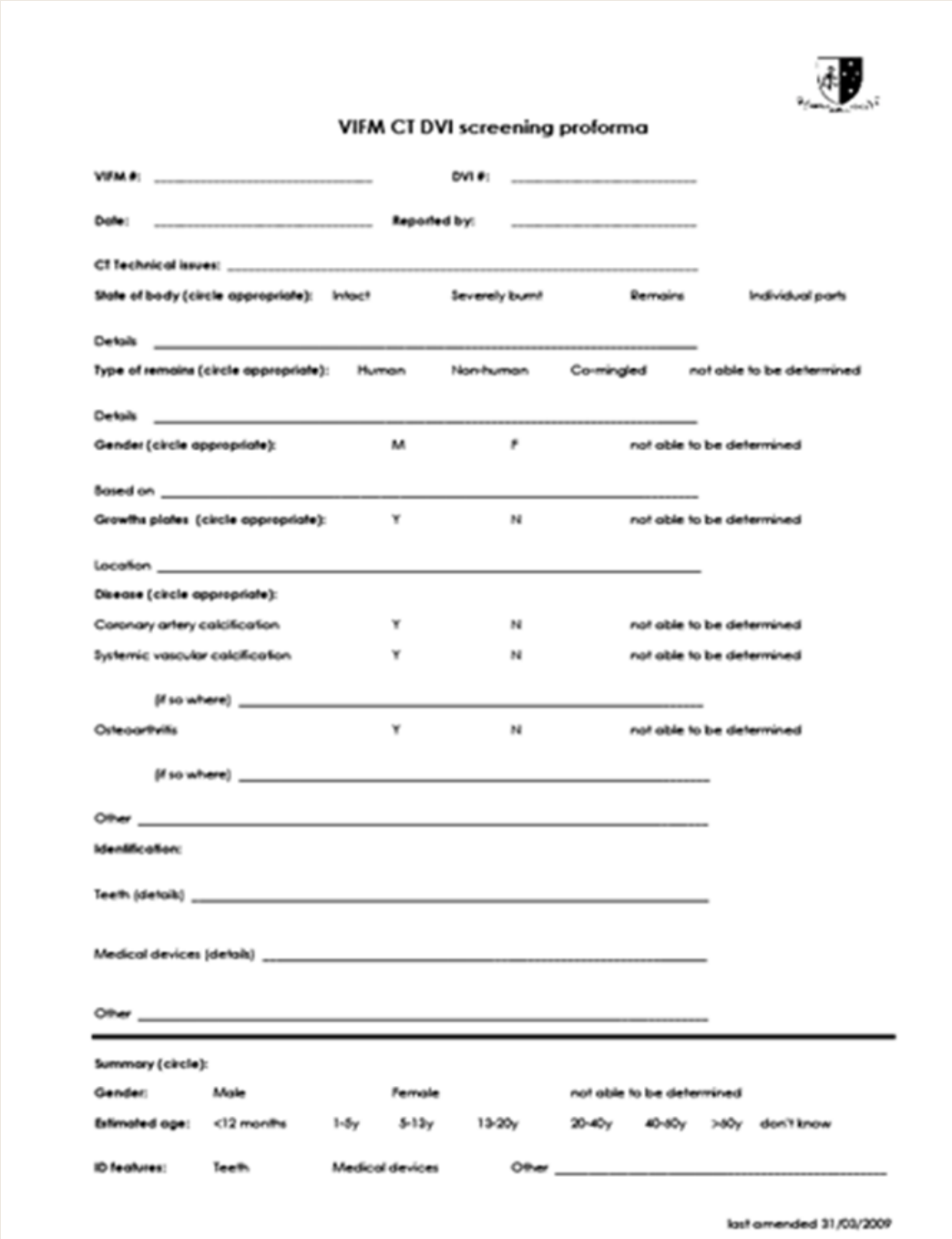


VIFM CT DVI screening proforma

VIFM #: _____ DVI #: _____

Date: _____ Reported by: _____

CT Technical issues: _____

State of body (circle appropriate): Intact Severely burnt Remains Individual parts

Details _____

Type of remains (circle appropriate): Human Non-human Co-mingled not able to be determined

Details _____

Gender (circle appropriate): M F not able to be determined

Based on _____

Growths plates (circle appropriate): Y N not able to be determined

Location _____

Disease (circle appropriate):

Coronary artery calcification Y N not able to be determined

Systemic vascular calcification Y N not able to be determined

(if so where) _____

Osteoarthritis Y N not able to be determined

(if so where) _____

Other _____

Identification:

Teeth (details) _____

Medical devices (details) _____

Other _____

Summary (circle):

Gender: Male Female not able to be determined

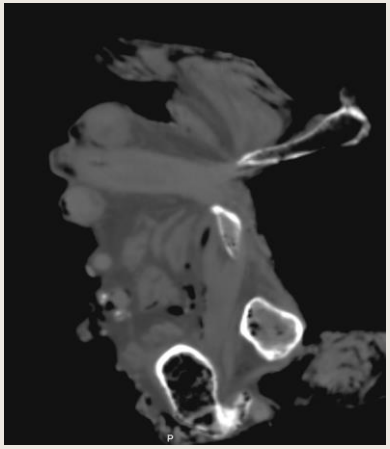
Estimated age: <12 months 1-5y 5-13y 13-20y 20-40y 40-60y >60y don't know

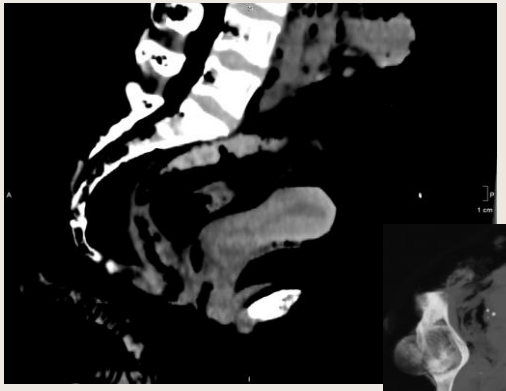
ID features: Teeth Medical devices Other _____

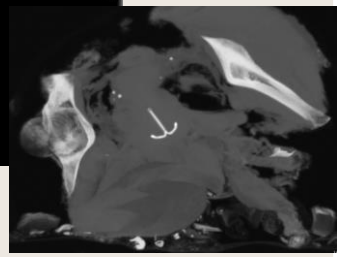
last amended 31/03/2009

ID

Sex (non-anthropological)







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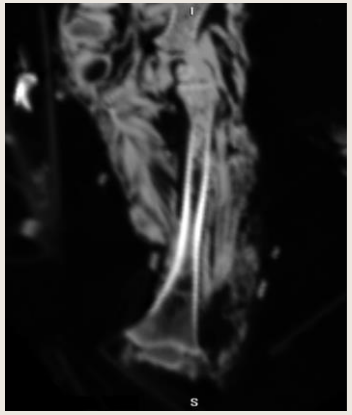
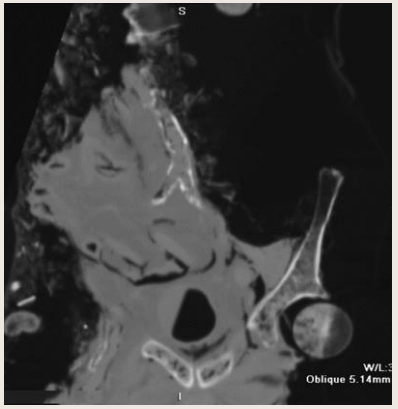
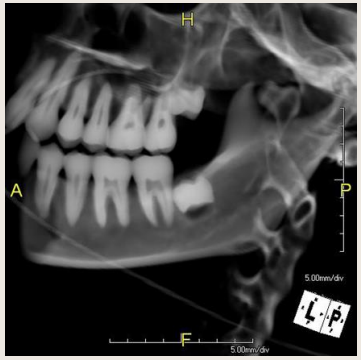
ID

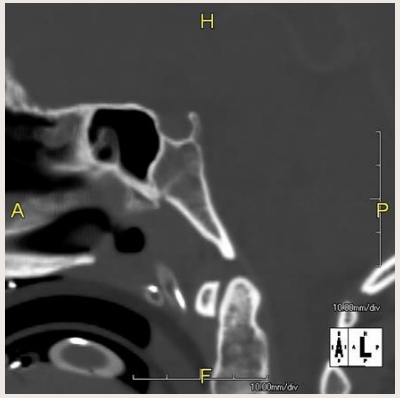
Age estimation (non-anthropological)





AGE ESTIMATION
MULTI-MODALITY





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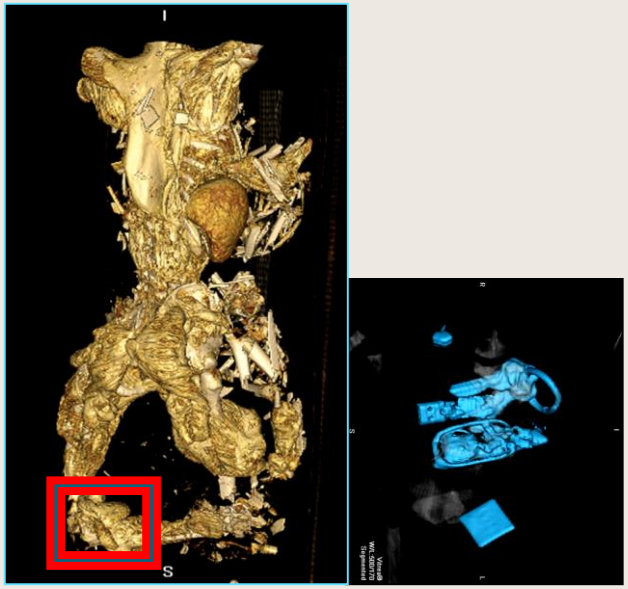
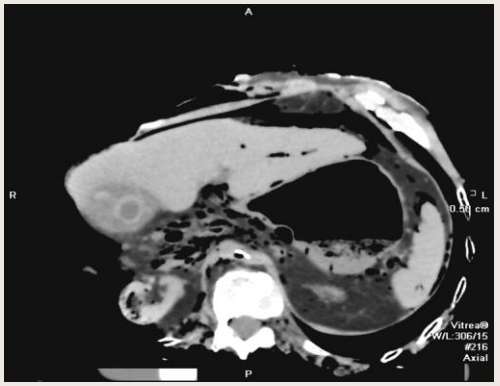
ID

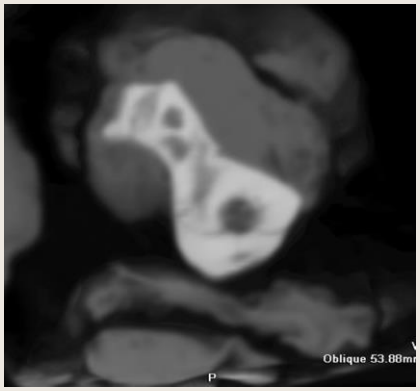
Jewellery and other objects

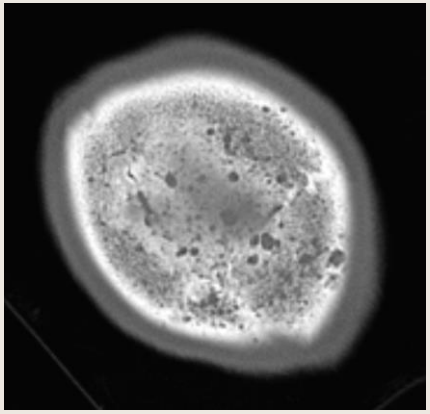


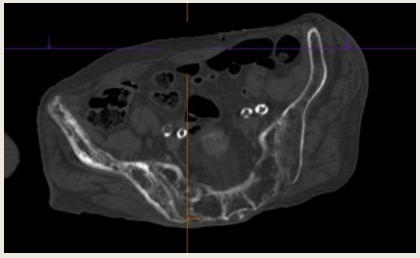
ID

Disease/deformity









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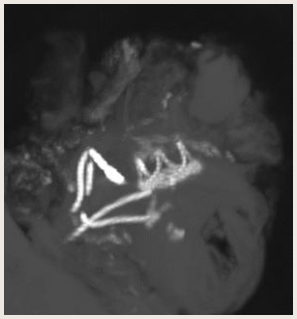
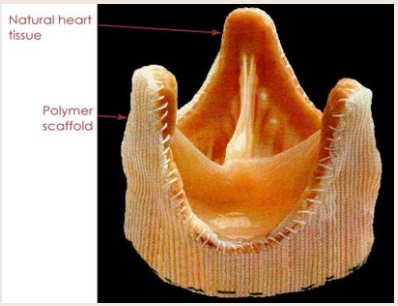
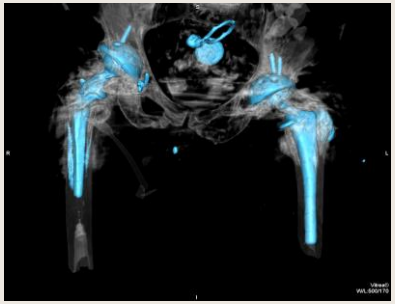
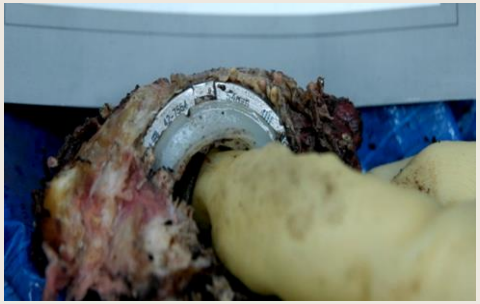
ID

Medical devices









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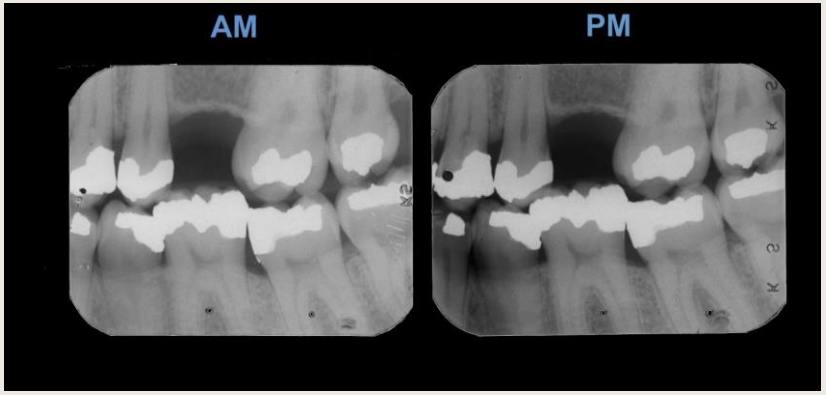
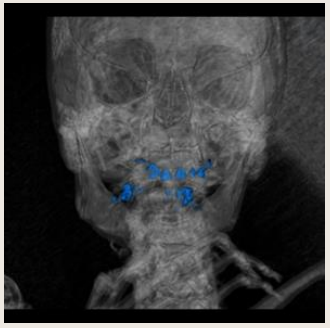
ID

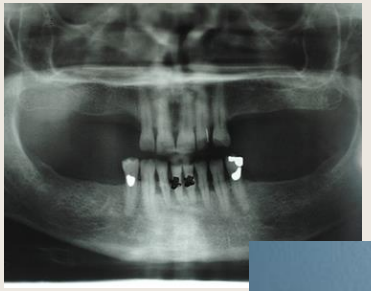
Dental- Plain Xray



RADIOLOGY

Dental- CT





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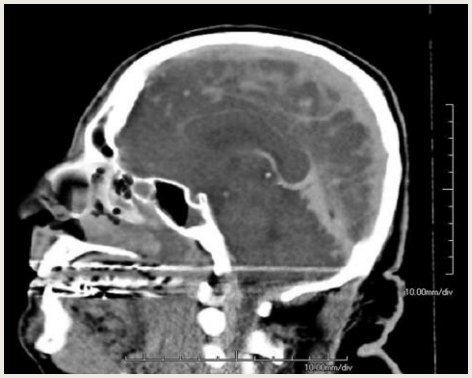
PROBLEMS

- Artefacts
- Positioning- non orthogonal
- Small fragments/building materials
- Expensive*
- Limited portability/availability
- Radiological/radiographic expertise
- Servicing
- Data Storage- PACS



CT RADIOLOGY SUMMARY

Rapid processing of remains

Permanent record

Supplements physical examination

Minimise tissue loss/hazards

Aid in primary and secondary identification

Can be resource intensive- money, personnel

MOLECULAR BIOLOGY

NEW APPROACHES

MOLECULAR BIOLOGY APPLICATIONS

Routine identification:

- Nuclear DNA (nDNA) – 16 autosomal markers including sex determination
- Mitochondrial DNA (mtDNA)

Disaster Victim Identification (DVI):

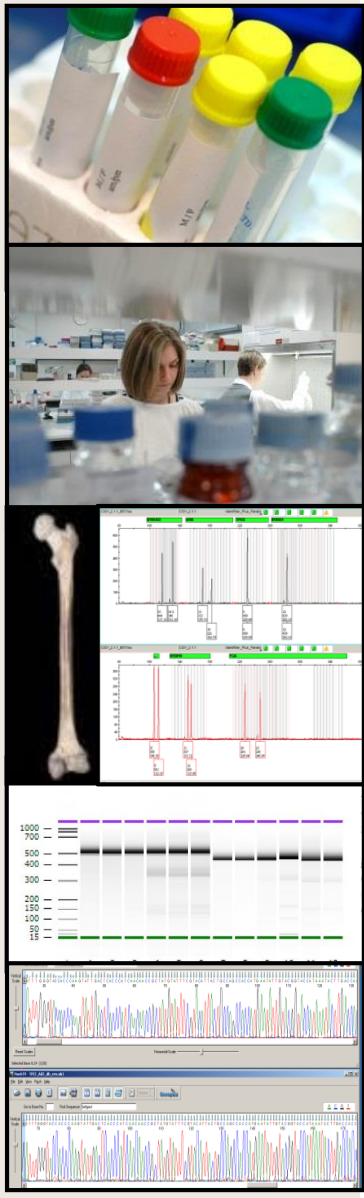
- Multiple fatalities- few to hundreds

Missing persons investigations:

- Unidentified remains reconciled with missing persons

DNA testing in old specimens:

- mtDNA analysis



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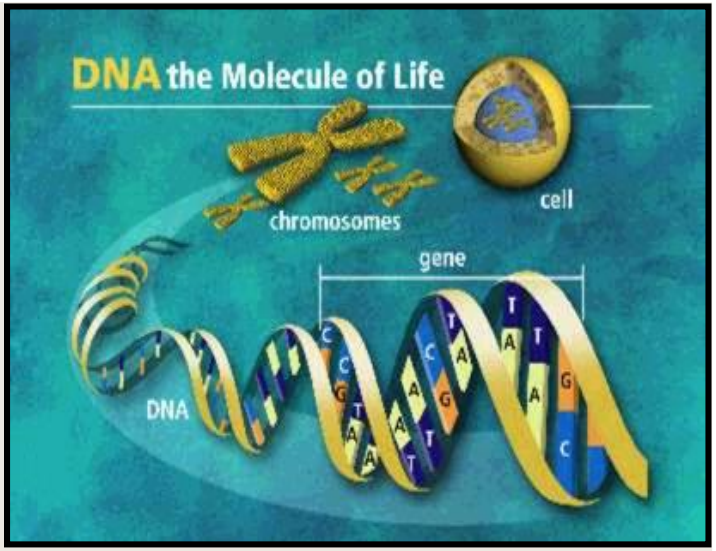
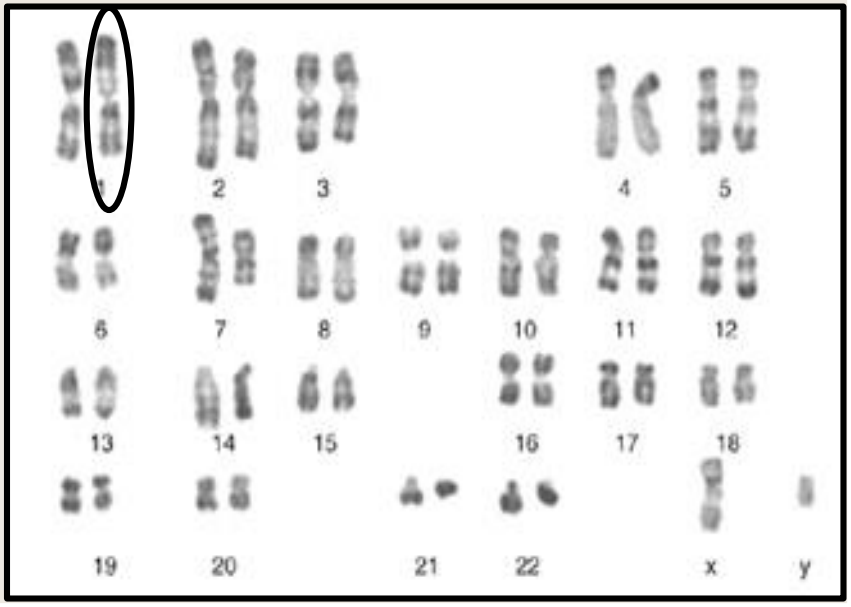
NUCLEAR DNA (nDNA)



- Nucleus (one per cell)
- One copy of nDNA per cell
- Large
- Packaged into structures
  - Chromosomes
    - 23 pairs of chromosomes in a human cell
    - Including the sex-determining X and Y chromosomes
- Mode of inheritance
  - ½ from mother
  - ½ from father



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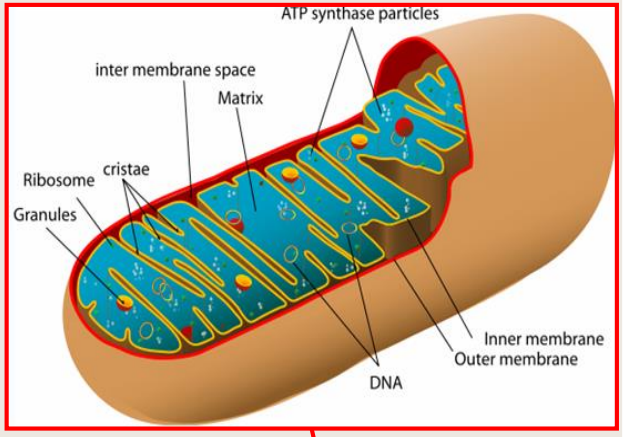
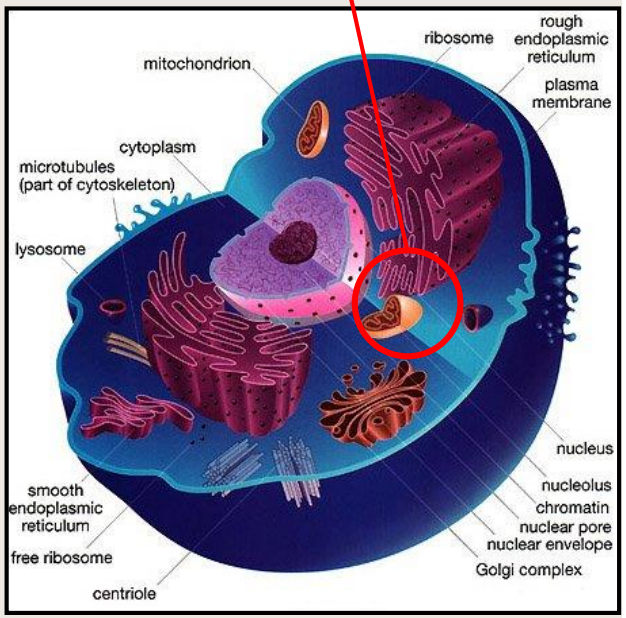
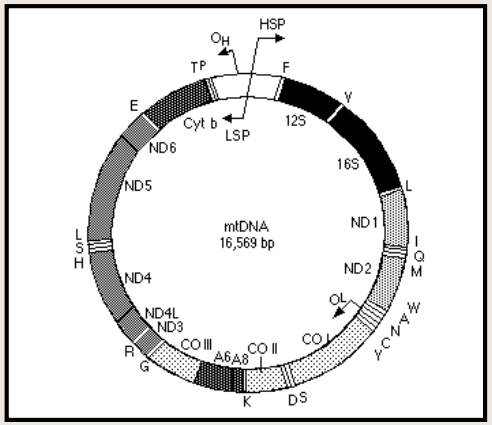
MITOCHONDRIAL DNA (mtDNA)

- Small
- Circular genome
- Mitochondria (many per cell)
- Several copies mtDNA per mitochondria
- 100s copies per cell
- *Relatively stable* – compartmentalisation
- More mtDNA compared to nDNA
- mtDNA is exclusively inherited from the mother







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DNA IDENTIFICATION

Kinship:

- nDNA analysis
- Compare profiles to establish if individuals are related

  -Parent/child relationships

  -Sibship (same parents)

Direct comparison with ante-mortem data:

- Self to self (e.g. deceased compared to Guthrie cards, histology blocks, hair etc.)

Challenges:

- Incinerated remains
- Decomposed remains

INCINERATED REMAINS

Range of body types

- Intact charred remains to fragmented burnt and calcined bones

Varied success of DNA analysis- 2009 experience

- Good for bone/ muscle/ blood from charred remains
- Poor from fragmented burnt bones

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DNA TRIAGE PROCESS- 2009 FIRES

Post-mortem sample collection

Condition of body	Sample to be collected
Not decomposed, whole body	Blood (on FTA card or swab) and buccal (mouth) swabs
Not decomposed, fragmented	If available, blood And Deep red muscle tissue (~1.0 g)
Decomposed, whole bodies and fragmented remains	Long compact bone samples (cut 4–6 cm, using window cut without separating the shaft) And/or Healthy teeth without fillings (molars preferable) And/or Any available bone (~10 g, if possible; dense cortical bone preferable)
Severely burnt bodies	Any of the samples above Or Swab from inside the urinary bladder (see Ref. [32])

Reference: Prinz, M. et al (2007) FSI Genetics (1) 3-12

FSI Genetics (1) 3-12

2011 PNG FLIGHT 1600: 28 DECEASED

- Variation in preservation- many victims severely burnt
- Bladder preserved intact
- Bladder swabs collected for DNA analysis in addition to routine specimens- AFP
- Full DNA profiles obtained from all samples
- ? Applicability to routine case work- research

BLADDER SWABS: SAMPLE COLLECTION PROTOCOL

Standard sample

- Dependent on the degree of incineration- bone, blood, muscle

Bladder swab sample

- Small incision (~1 cm) in the anterior wall of the bladder
- Dry cotton swab inserted
- Bladder wall wiped
- If delay (>12 hrs) – aeration required

RESEARCH- BLADDER SWABS 2013

- All fire deaths admitted to SCO- January- November 2012
- House fires; car accidents; aviation; self immolation; homicide.
- 28 cases- wide variability in preservation
- Routine specimens for comparison- blood, muscle, bone depending on case

BLADDER SWAB RESULTS

- nDNA extracted regardless of condition of swab (yellow to red)
- Extraction techniques- almost identical to buccal swabs. Easy and robust
- 95% of bladder swabs showed greater nDNA yields (compared with blood or muscle)
- 2 cases showed lower nDNA yields (compared with bone)- still adequate for ID
- Overall 1-10x more DNA from bladder swab samples

BLADDER SWAB STUDY- CONCLUSIONS

- Bladder swabs are a reliable source of DNA for STR analysis
- Ideal for IDs involving incinerated cases
- Minimally invasive techniques
- Simple extraction, good DNA yield
- Reduce the time and complexity in identification.

Sample type	Sample preparation (hours)	Results available (days)
Bone	6-12	2
Muscle tissue	2-6	1-2
blood	1	1
Bladder swab	1	1

DECOMPOSED CASES- TOENAIL DNA RESEARCH- 2016

- Typical sample- compact bone or head of femur
- Previous focus on nail scrapings in criminal investigations- few ID studies
- Nails similar to bone- hard material resistant to environmental damage and decomposition
- ?Toenails- lower numbers of mixed profiles
- Easily accessible- minimal training required
- Decreased processing time

- Study to develop and validate technique for nail analysis

TOENAIL DNA RESEARCH

30 decomposed cases 2013-14

- **Average PM interval 3 weeks (2 days to 9 months)**
- **unsuitable for visual ID**
- **toenail + conventional sample (bone)**

Extraction methods optimised (e.g. washing and digestion times) by parallel live donor study (buccal swab and toenail clippings- 46 cases)

- **Adaption of hair extraction technique**
- **2 methods validated – silica based column purification (Qiagen) and organic (lab)**
- **> 0.01g nail material required for full profile**

RESULTS

Volunteer specimens

- **Both methods yielded sufficient DNA for ID purposes**
- **Optimized Qiagen method better for more complete profile**

Decomposed cases

- **Required additional decontamination step (scraping)**
- **Overall, toenails comparatively more degraded than bone**
- **nDNA extracted from all 30 toenail cases**
- **2/30 bone samples failed to produce adequate nDNA**
- **38% of toenail cases produced higher yield than bone in the same case**

CONCLUSION- BENEFITS OF USE OF TOENAIL MATERIAL

- Significant reduction in sample preparation time - 2 hours as compared with 6-12 hours for bone and 2 – 6 hours for muscle
- Reduced occupational health and safety risks for staff
- Less invasive/technically demanding + faster sample collection (15’ v 2’)
- Faster overall processing time
- Easier to store (smaller samples, no refrigeration)

PUBLICATIONS

Forensic Science International 233 (2013) 14–20

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Post mortem sampling of the bladder for the identification of victims of fire related deaths

Rebecca Owen [a], Paul Bedford [a], Jodie Leditschke [a,b], Andrew Schlenker [a], Dadna Hartman [a,b,*]

[a] Victorian Institute of Forensic Medicine, 57-83 Kavanagh Street, Southbank, VIC 3006, Australia
[b] Department of Forensic Medicine, Monash University, Australia

Forensic Science International 258 (2016) 1–10

Contents lists available at ScienceDirect

Forensic Science International

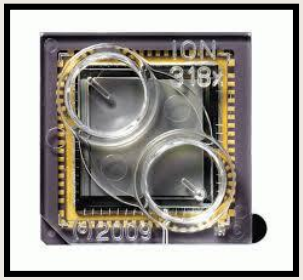
journal homepage: www.elsevier.com/locate/forsciint

Toenails as an alternative source material for the extraction of DNA from decomposed human remains

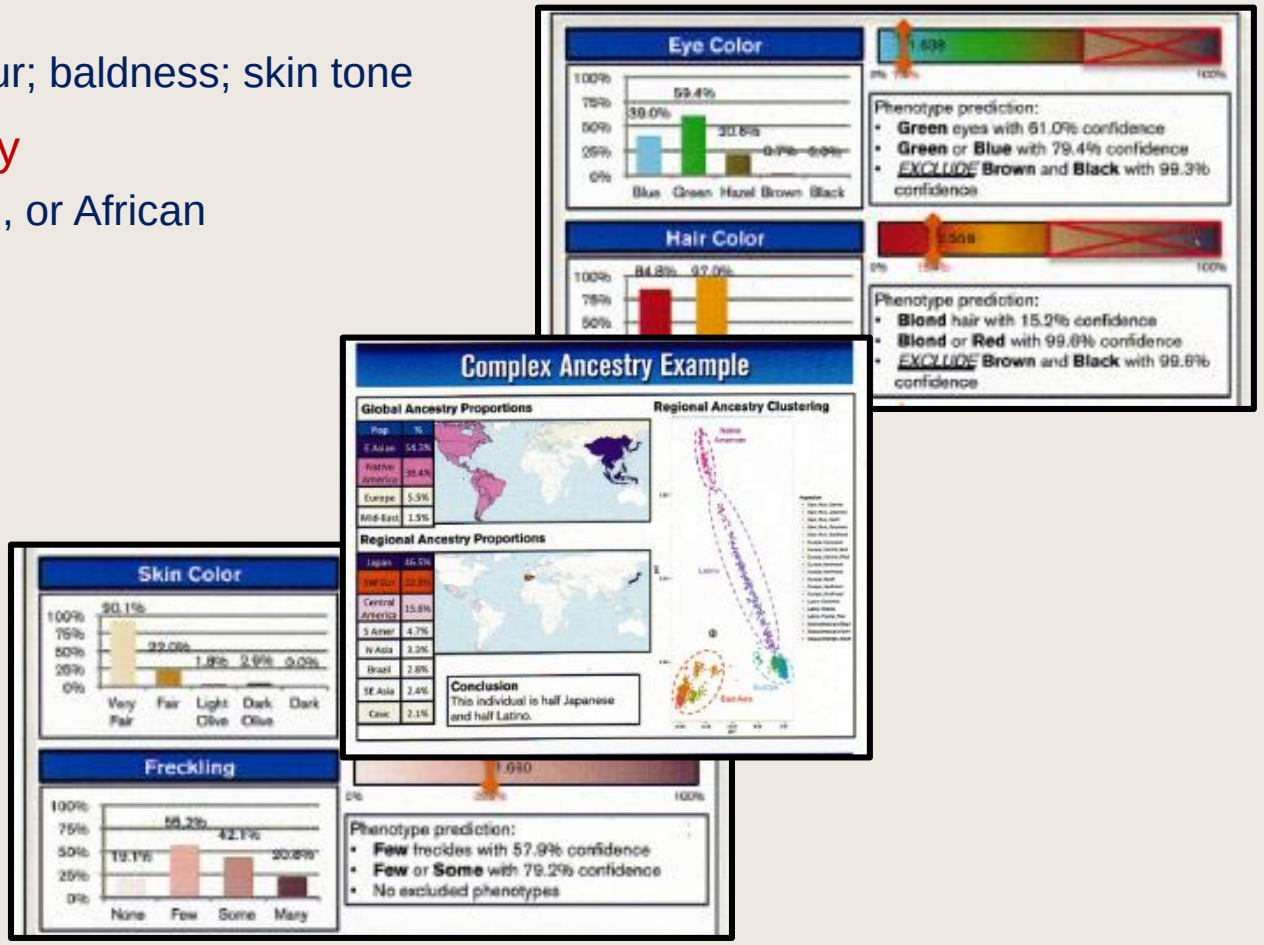
Andrew Schlenker [a], Katelyn Grimble [a], Arani Azim [a], Rebecca Owen [a], Dadna Hartman [a,b,*]

[a] Victorian Institute of Forensic Medicine, 65 Kavanagh St, Southbank, VIC 3006, Australia
[b] Department of Forensic Medicine, Monash University, Australia

WHERE TO FROM HERE?



- **Massive parallel sequencing (MPS)**
  - Determine the DNA sequence of many (1000s) DNA fragments at once
- **DNA sequences that predict physical appearance**
  - Phenotypic features
    - Eye colour; hair colour; baldness; skin tone
  - Geographical ancestry
    - E.g. European, Asian, or African



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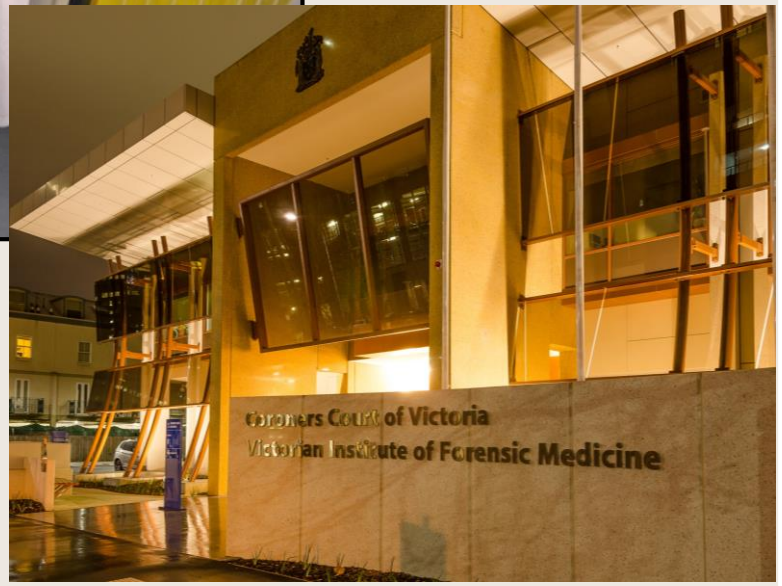
VIFM MOLECULAR BIOLOGY LABORATORY STAFF



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ISFRI 2018

10-12 May 2018
Melbourne Australia

ISFRI | International Society of Forensic Radiology and Imaging

www.isfri2018.com

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QUESTIONS?

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