



**BEGINNING SHORTLY**

**VIETNAM WAR**

**ANNUAL GOVERNMENT BRIEFING**



DEFENSE POW/MIA ACCOUNTING AGENCY

# Vietnam War Annual Government Briefing

Omaha, NE

*26-27 June 2025*



DEFENSE POW/MIA ACCOUNTING AGENCY

# Opening Comments

**Mrs. Jennifer Nasarenko**

**Director, Outreach & Communications**

*Vietnam War Annual Government Briefing 26-27 June 2025*

# **A VSO's View**

**Mr. Gene Murphy, DAV Treasurer, Department of South Dakota**

**Mr. Brian Walker, VFW Adjutant General**

**Mr. Grant Coates, VVA POW/MIA Committee Chair**

*Vietnam War Annual Government Briefing 26-27 June 2025*



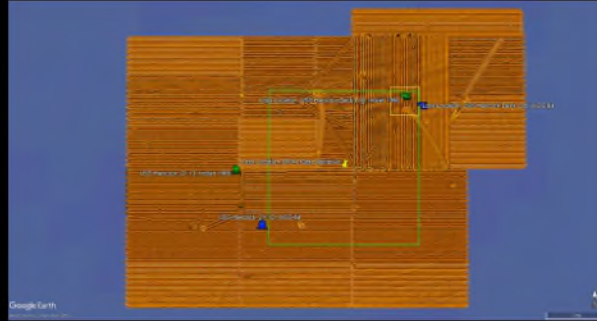
The Defense POW/MIA Accounting Agency and  
Scripps Institution of Oceanography Underwater  
Missions in the Gulf of Tonkin 2020 - 2024



Coastal Observing  
Research & Development Center



# SURVEY TIMELINE



2020

2022

2023

2024

- B-52 Stratofortress
- REFNO 0757/0758
- 10 Day Survey
- 3 AUVs
- 28.5 km<sup>2</sup> survey
- 1 aircraft site discovered
- Remains of 1 MIA recovered

- CH-46D Sea Knight
- REFNO 2024
- 12 Day Survey
- 3 AUVs
- 74 km<sup>2</sup> survey
- No site discovered

- A-6A Intruder
- REFNO 0148
- S-2D Tracker
- REFNO 0237
- 12 Day Survey
- 3 AUVs
- 1 ROV
- 48.5 km<sup>2</sup> survey
- Discovered 1 aircraft site

- A-6A Intruder
- REFNO 0148
- 12 Day Survey
- 3 AUVs
- 1 ROV
- 22 km<sup>2</sup> survey
- Discovered 1 aircraft site

Wide-Area Search

**Find**



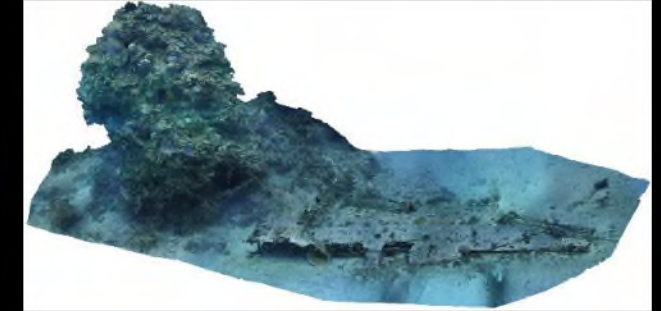
Target Reacquire

**Fix**



Target Documentation

**Finish**



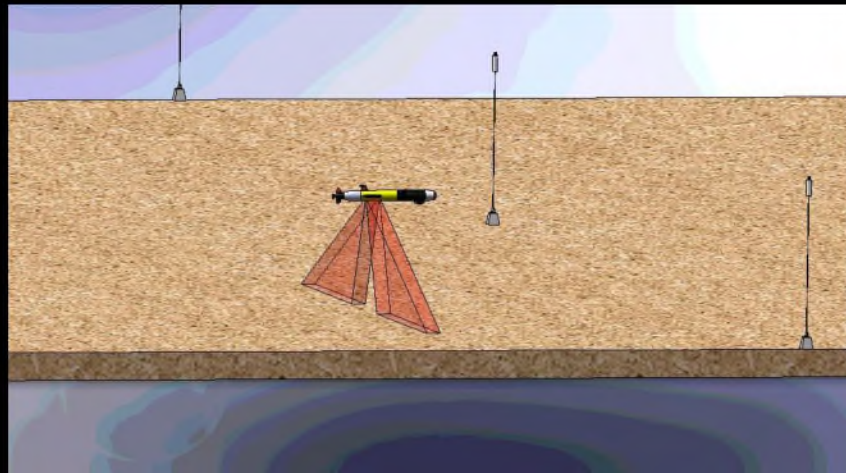
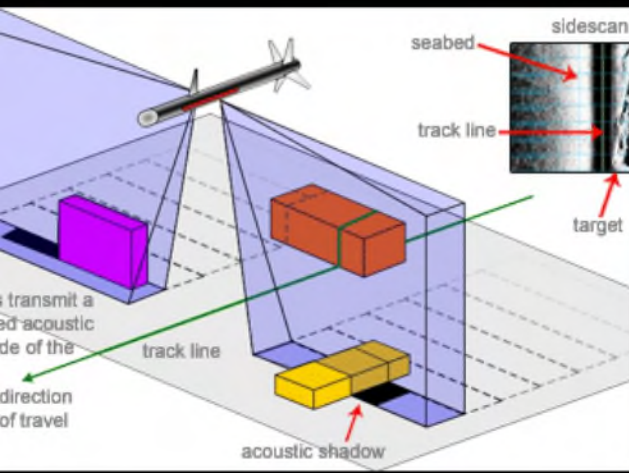
Sensor	FIND	FIX	FINISH
UUV REMUS 100 sidescan sonar 600 kHz	✓	•	•
Vessel-based multibeam (T50)	✓		•
UUV REMUS 100 magnetometer	✓	✓	•
UUV REMUS 100 multibeam		✓	•
UUV REMUS 100 sidescan sonar 1200/1800 kHz	•	✓	✓
UUV REMUS 100 low light imager	•	✓	✓
Diver based navigator	•	✓	✓•
Barracuda ROV	•	✓	✓
Diver based digital camera	•		✓

Site Condition	UUV - SSS - Visible	ROV - Imaging Sonar - Visible	Multibeam Sonar	Sub Bottom Profiler	Diver - Imaging Sonar - Visible
Rocky low slope	✓✓✓	✓✓	✓✓✓	X	✓
Rocky high slope	✓	✓✓	✓✓	X	X
Sand	✓✓✓	✓✓	✓✓✓	✓	✓✓
Mud Buried or Partially	✓✓	✓✓	✓	✓✓✓	✓
Coral Reef low slope	✓✓	✓✓	✓✓✓	X	✓✓
Coral Reef high slope	✓	✓✓	✓✓	X	X
High Flow	✓	✓	✓✓✓	✓	X
Low Visibility	✓✓	✓✓	✓✓✓	✓✓✓	✓
>50 m	✓✓✓	✓✓✓	✓✓	✓✓	X

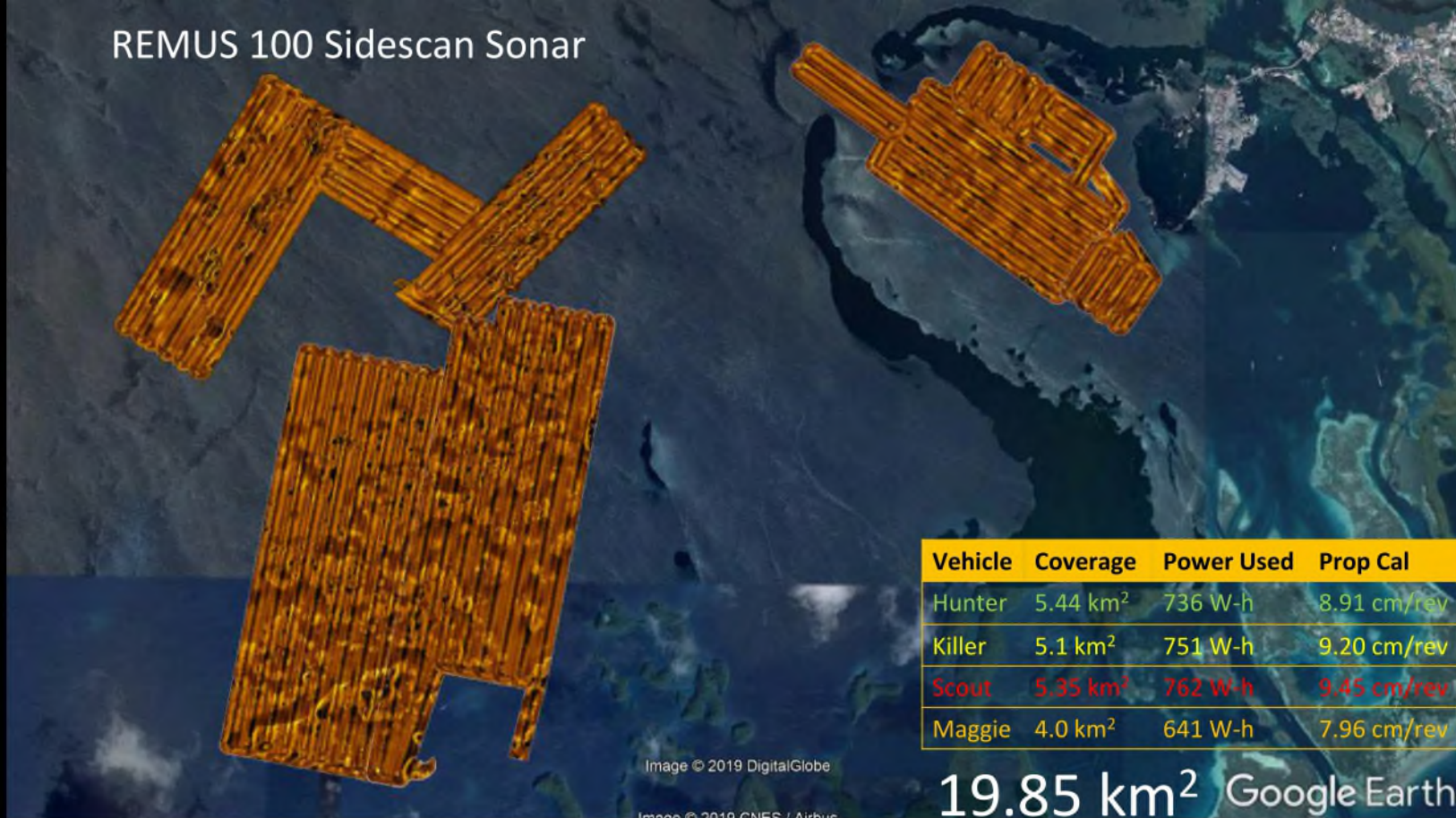


# REMUS Unmanned Underwater Vehicle

- *Precise navigation*
- *Sidescan sonar*
- *Underwater imaging*
- *Bathymetry, currents, water visibility, temperature*
- *100m depth rated*
- *2 man portable, small boat ops*



# REMUS 100 Sidescan Sonar



Vehicle	Coverage	Power Used	Prop Cal
Hunter	5.44 km <sup>2</sup>	736 W-h	8.91 cm/rev
Killer	5.1 km <sup>2</sup>	751 W-h	9.20 cm/rev
Scout	5.35 km <sup>2</sup>	762 W-h	9.45 cm/rev
Maggie	4.0 km <sup>2</sup>	641 W-h	7.96 cm/rev

19.85 km<sup>2</sup> Google Earth

Image © 2019 DigitalGlobe

Image © 2019 CNES / Airbus



Image Appearance

Digitized Features

Info at Cursor

10/09/2017 23:32:46

Ping: 240	WD:46.0 SD:0.0
04° 07.79648' S	
144° 53.12393' E	E:265251.63
HDG:186.60	CMG:194.85
S:70.0 H:66.5	SPD:3.50
ALT: 22.0	EVT: 0.00

Contrast: +/- 0

Gamma

Slant Range Corrected

Digitized Features

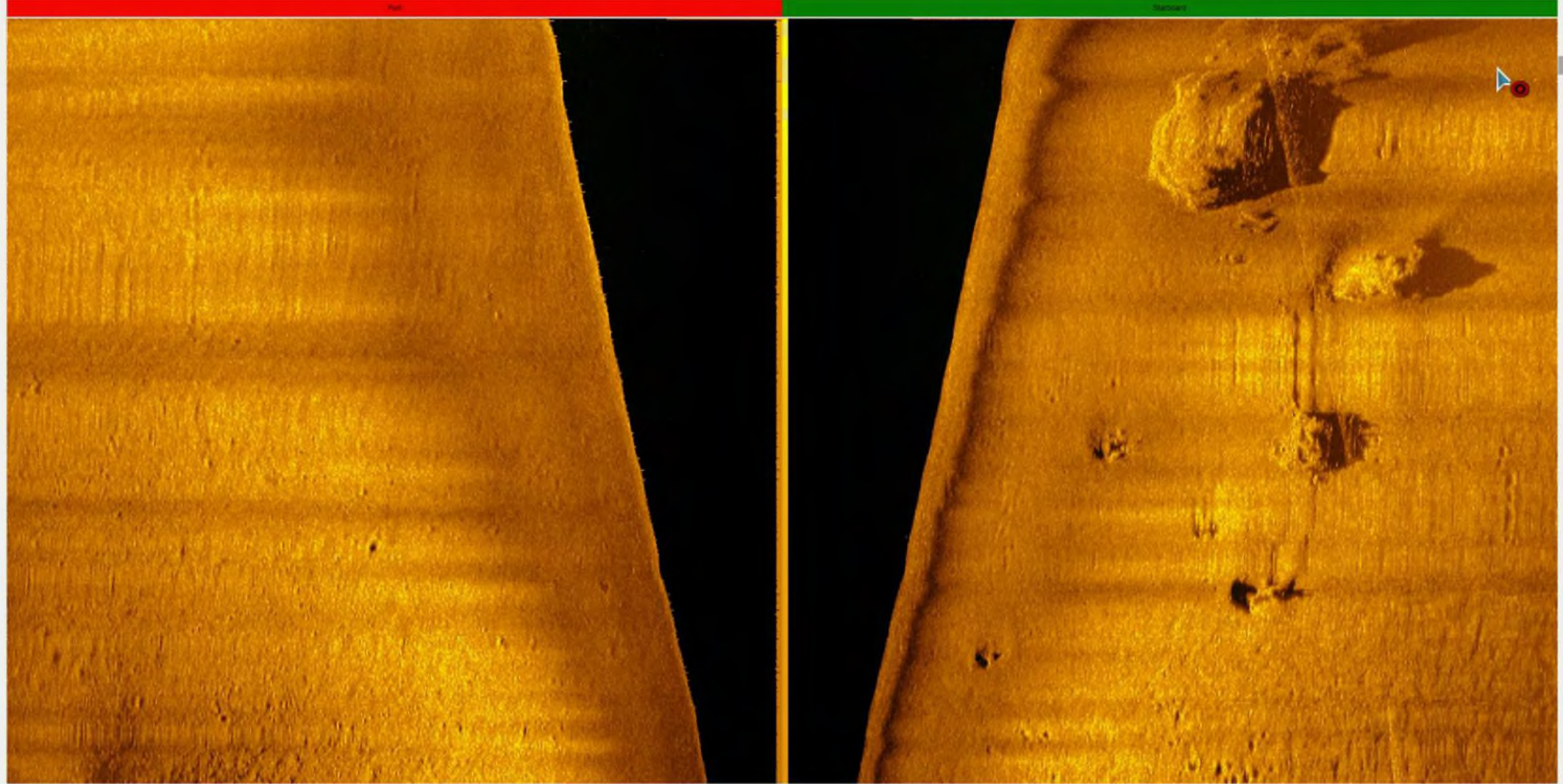
Contacts

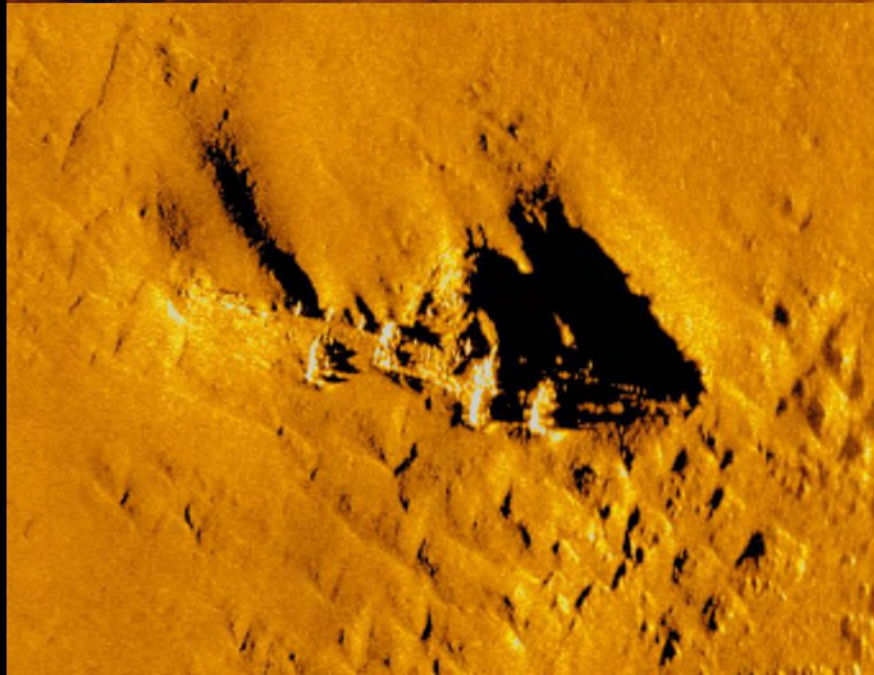
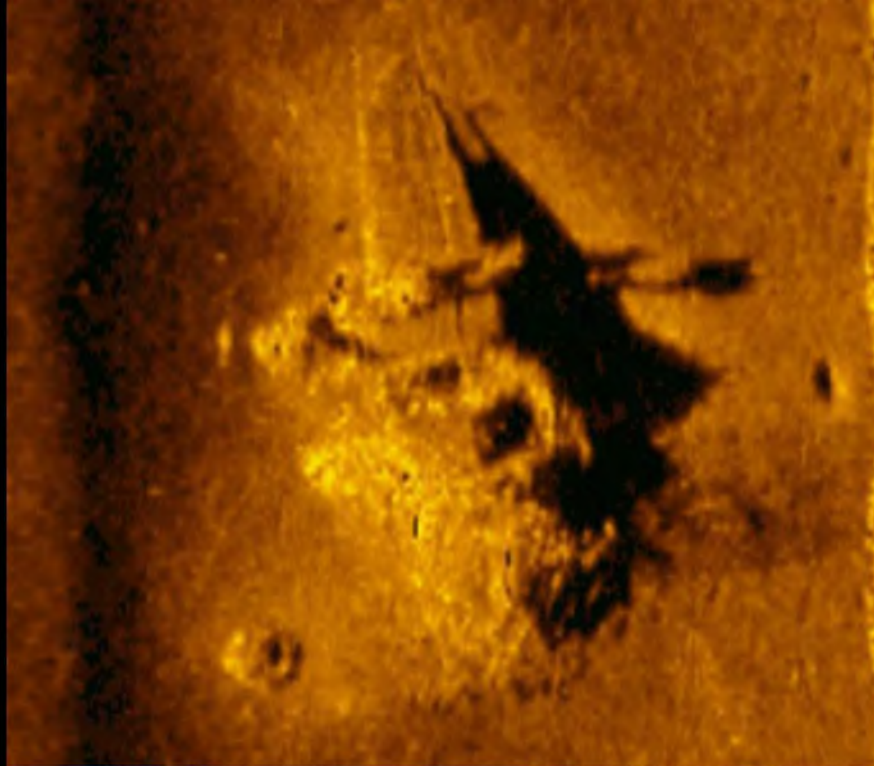
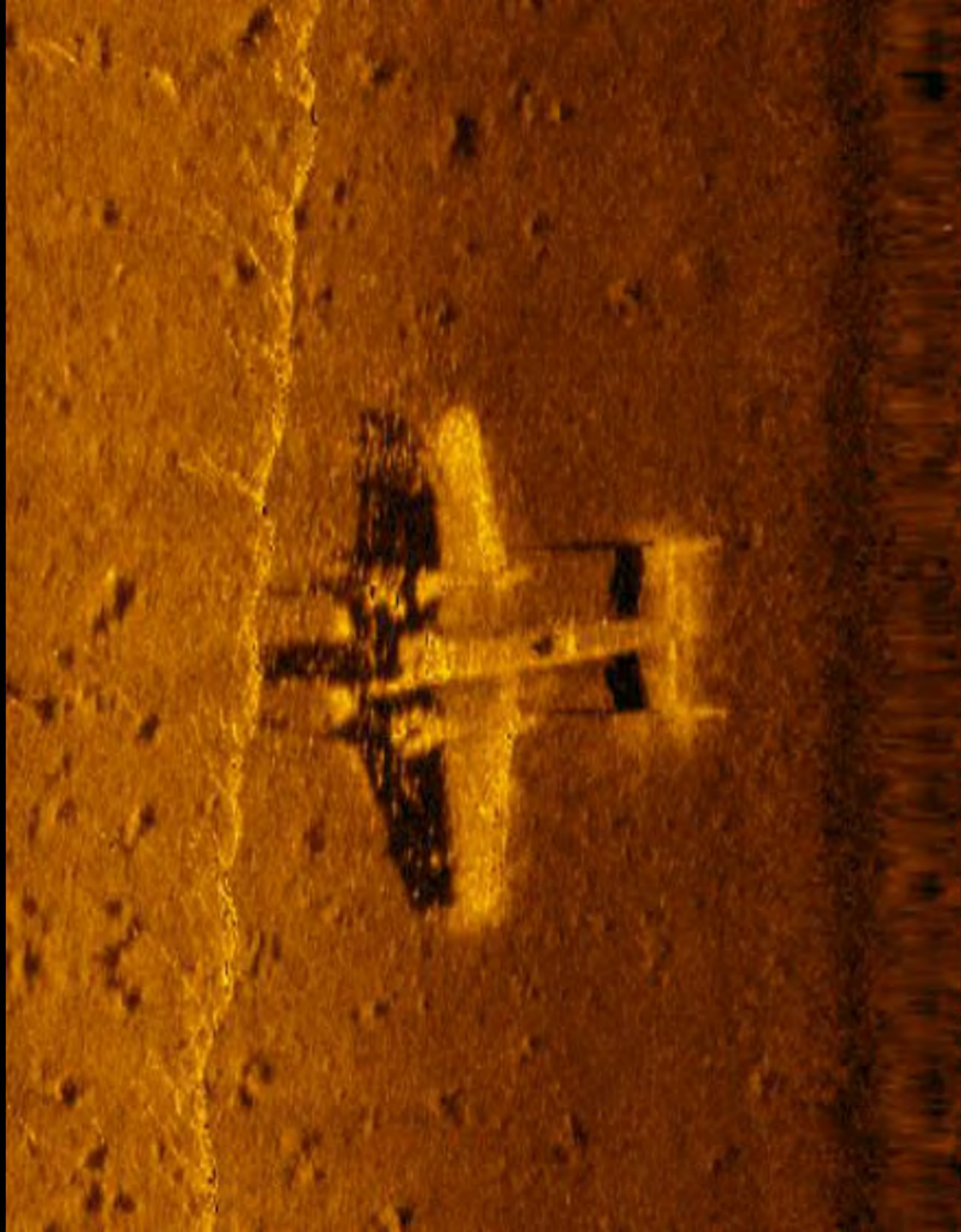
Options...

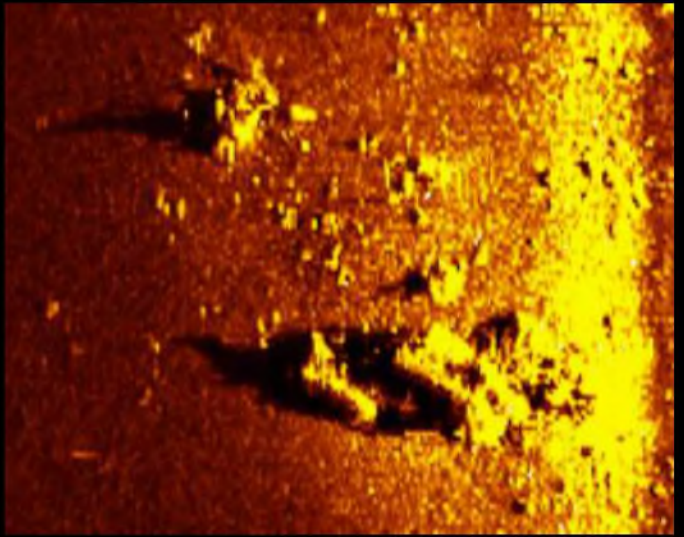
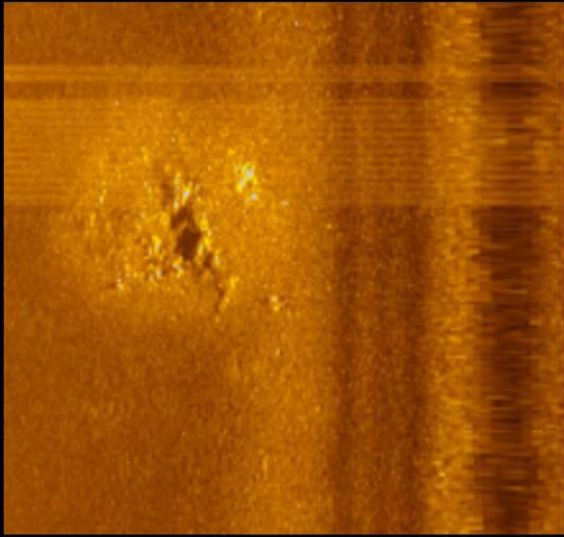
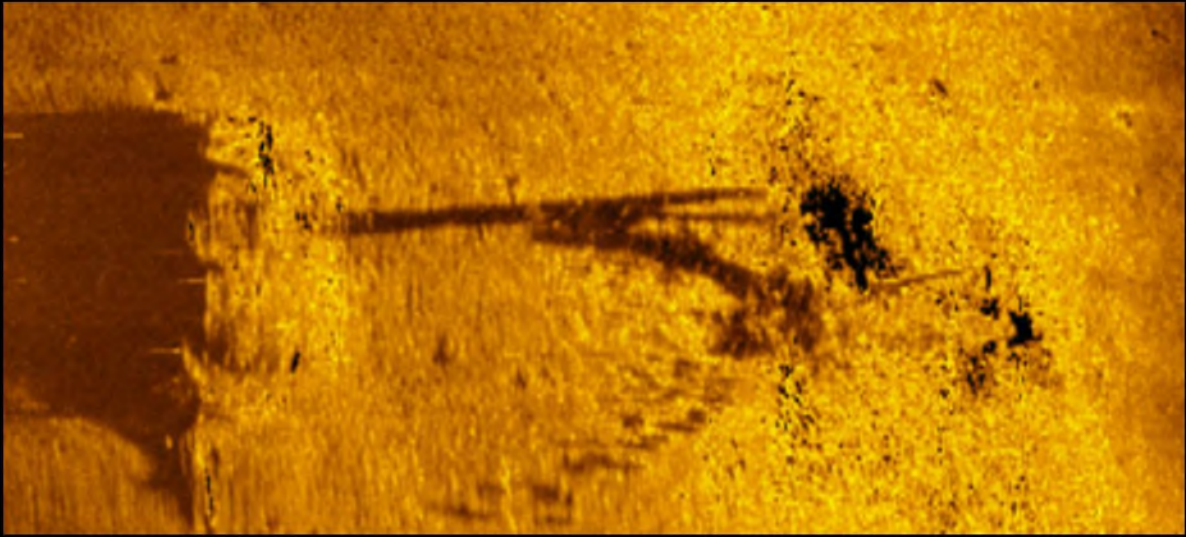
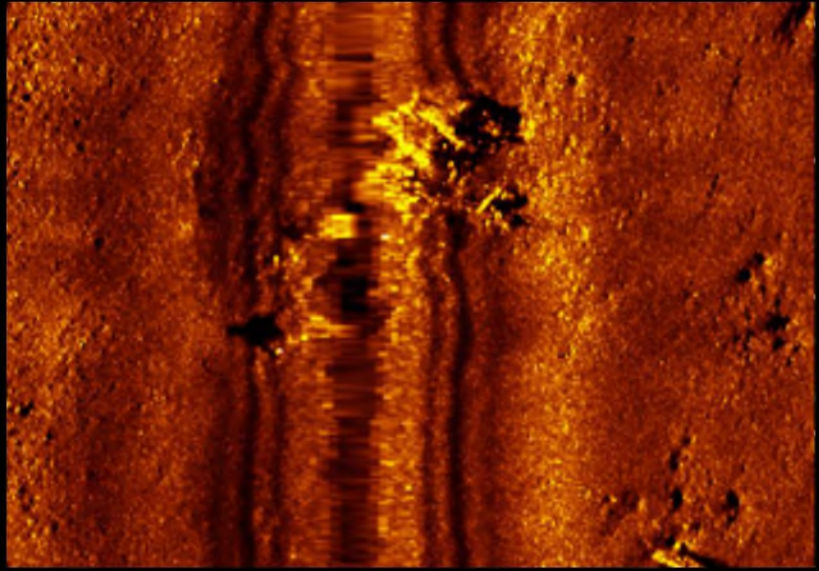
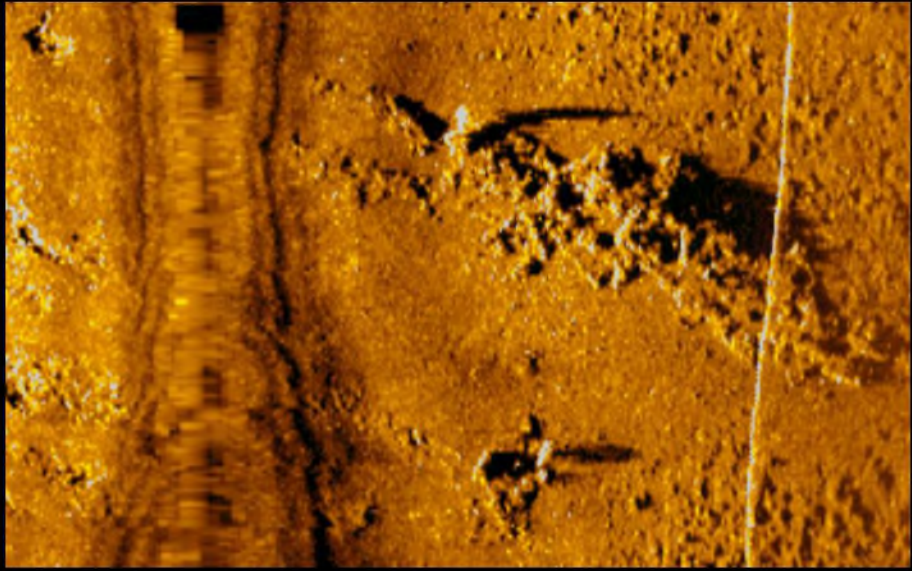
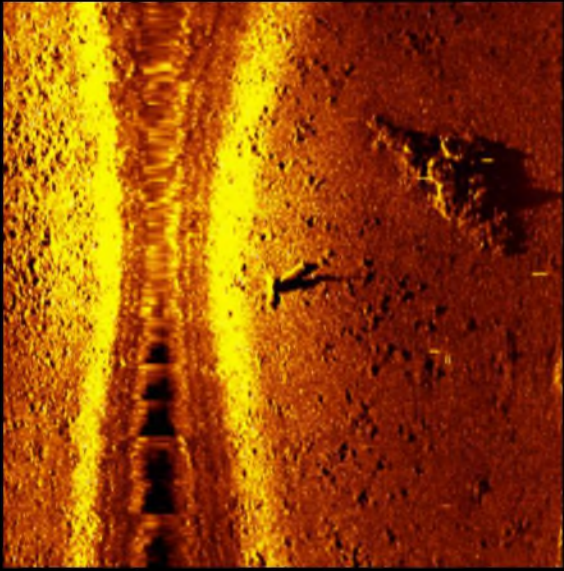
Split...

Use old non-virtual image control.

Fit Width OVERZOOM





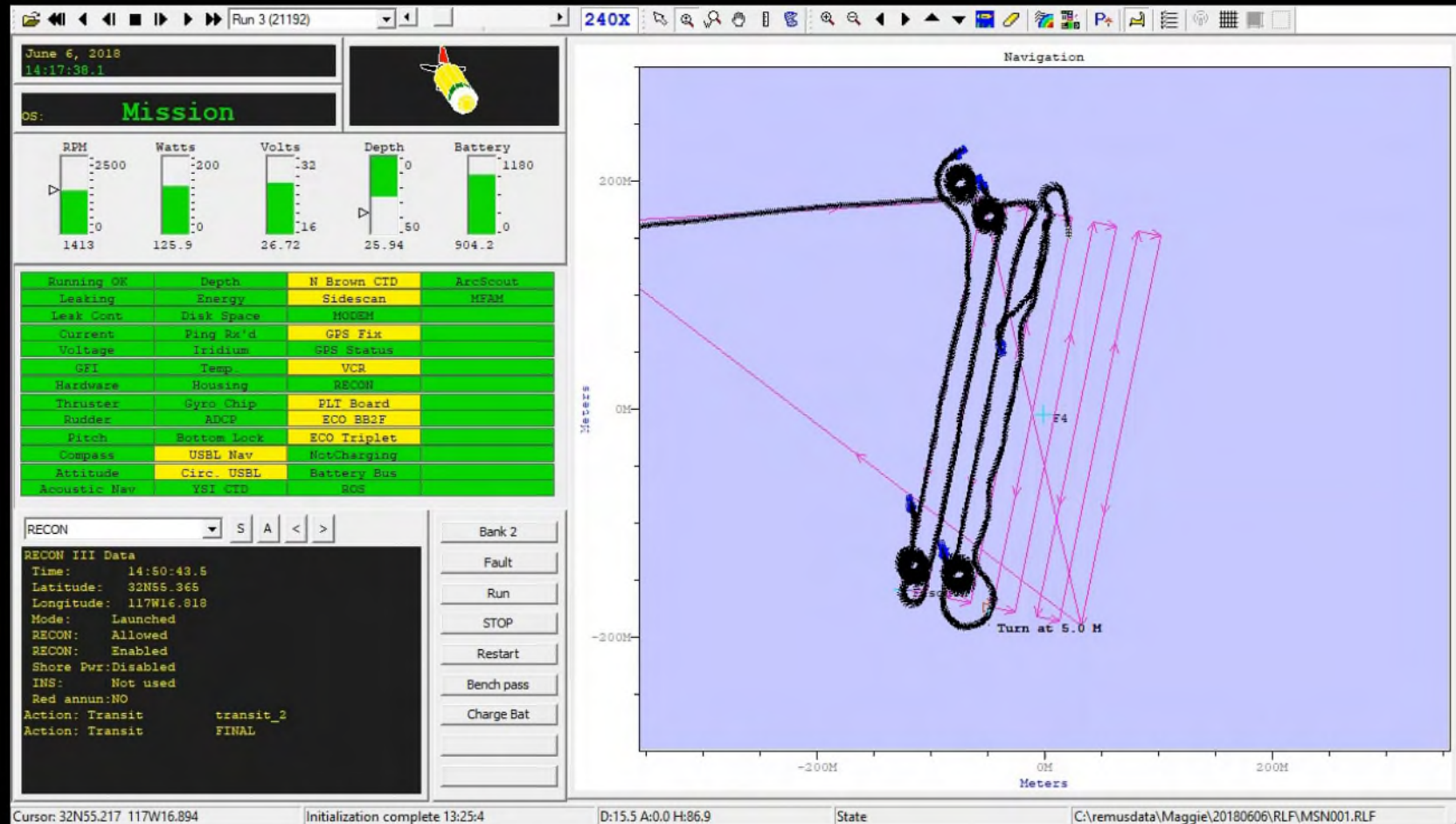
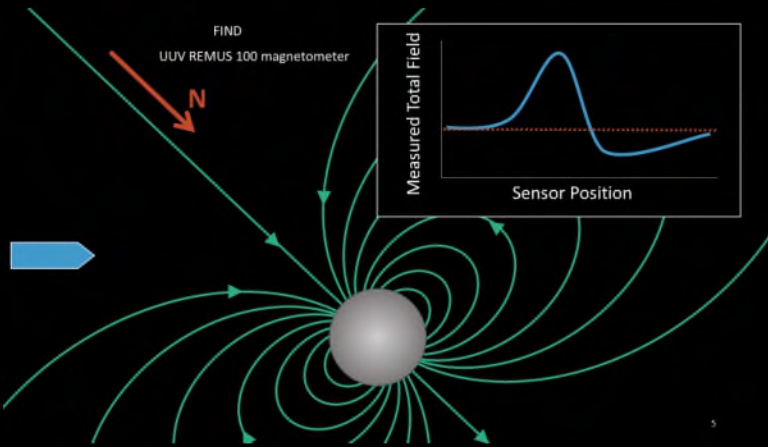


# FIND

MFAM = Micro Fabricated Atomic Magnetometer

SMALL FORM FACTOR MAG UNIT FOR UUV

# MFAM Payload for REMUS 100



Wide-Area Search

# Find



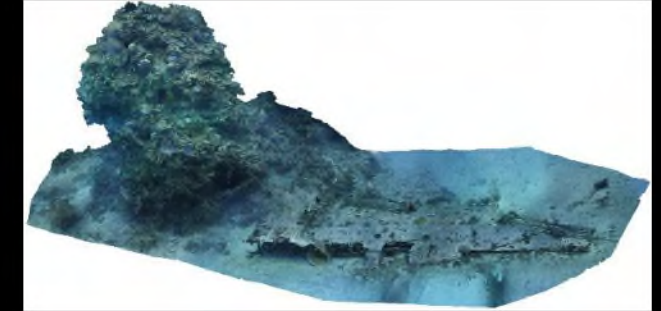
Target Reacquire

# Fix



Target Documentation

# Finish

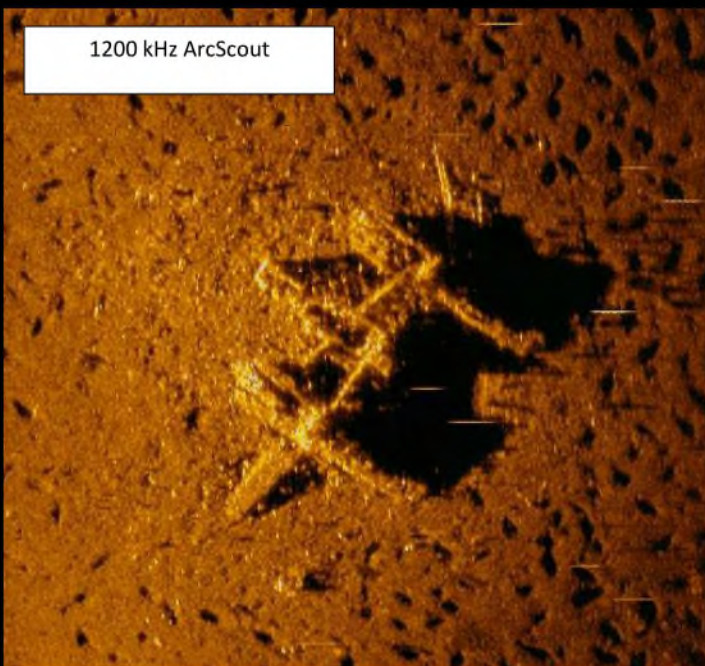
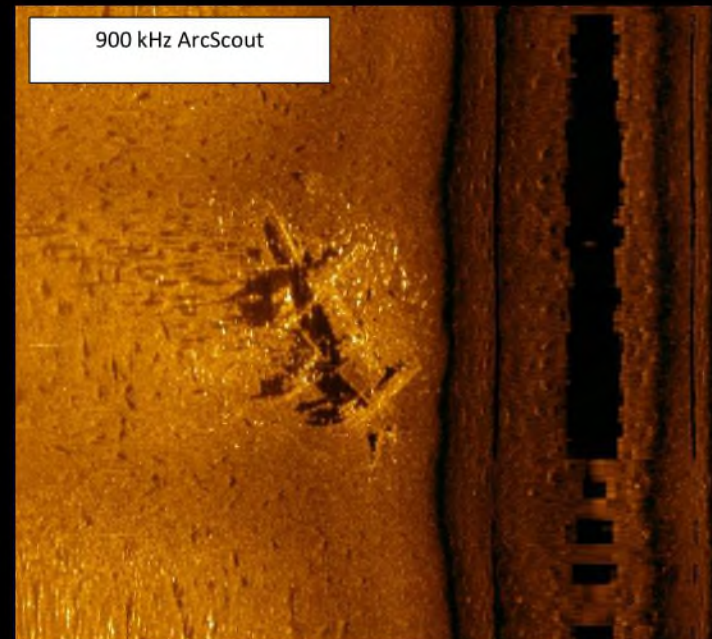
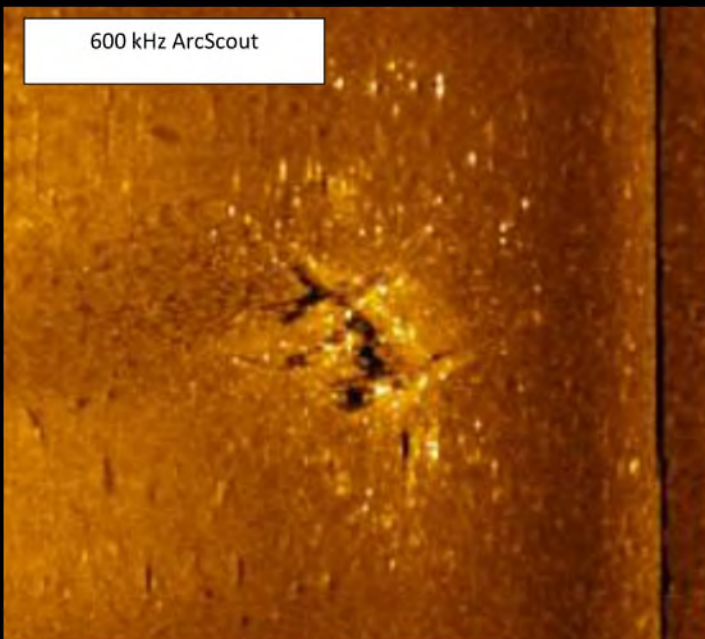


Sensor	FIND	FIX	FINISH
UUV REMUS 100 sidescan sonar 600 kHz	✓	•	•
Vessel-based multibeam (T50)	✓		•
UUV REMUS 100 magnetometer	✓	✓	•
UUV REMUS 100 multibeam		✓	•
UUV REMUS 100 sidescan sonar 1200/1800 kHz	•	✓	✓
UUV REMUS 100 low light imager	•	✓	✓
Diver based navigator	•	✓	✓•
Barracuda ROV	•	✓	✓
Diver based digital camera	•		✓

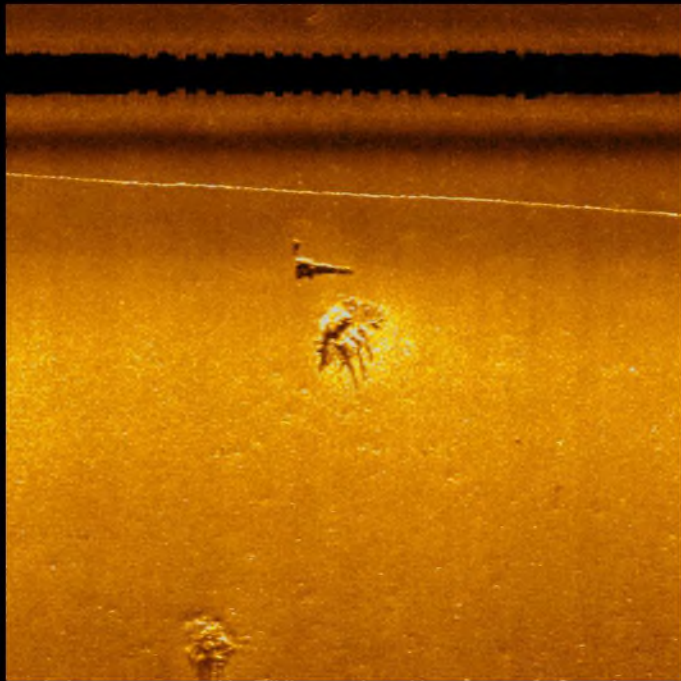
Site Condition	UUV - SSS - Visible	ROV - Imaging Sonar - Visible	Multibeam Sonar	Sub Bottom Profiler	Diver - Imaging Sonar - Visible
Rocky low slope	✓✓✓	✓✓	✓✓✓	X	✓
Rocky high slope	✓	✓✓	✓✓	X	X
Sand	✓✓✓	✓✓	✓✓✓	✓	✓✓
Mud Buried or Partially	✓✓	✓✓	✓	✓✓✓	✓
Coral Reef low slope	✓✓	✓✓	✓✓✓	X	✓✓
Coral Reef high slope	✓	✓✓	✓✓	X	X
High Flow	✓	✓	✓✓✓	✓	X
Low Visibility	✓✓	✓✓	✓✓✓	✓✓✓	✓
>50 m	✓✓✓	✓✓✓	✓✓	✓✓	X

# FIX

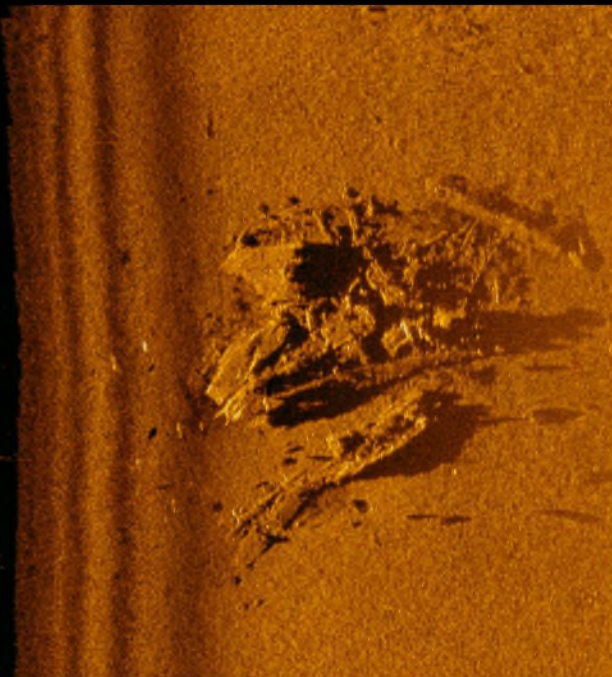
REMUS 100- 1200/1800 KhZ SSS



600 kHz

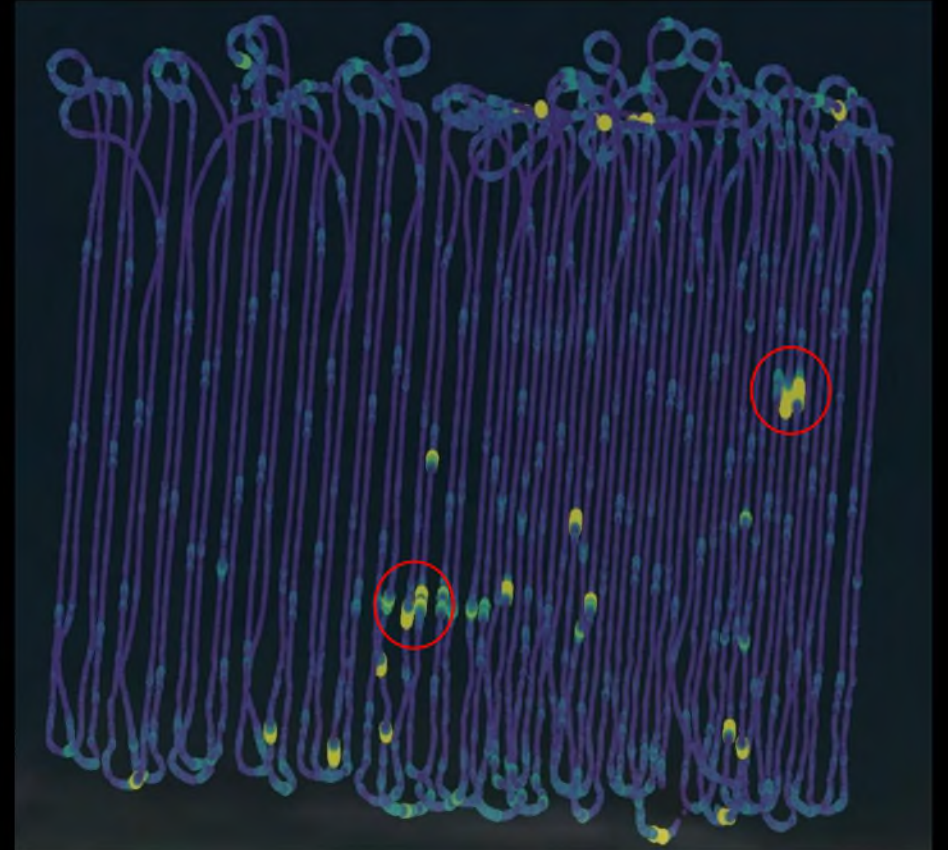


1800 kHz



# FIX

UUV REMUS 100 magnetometer



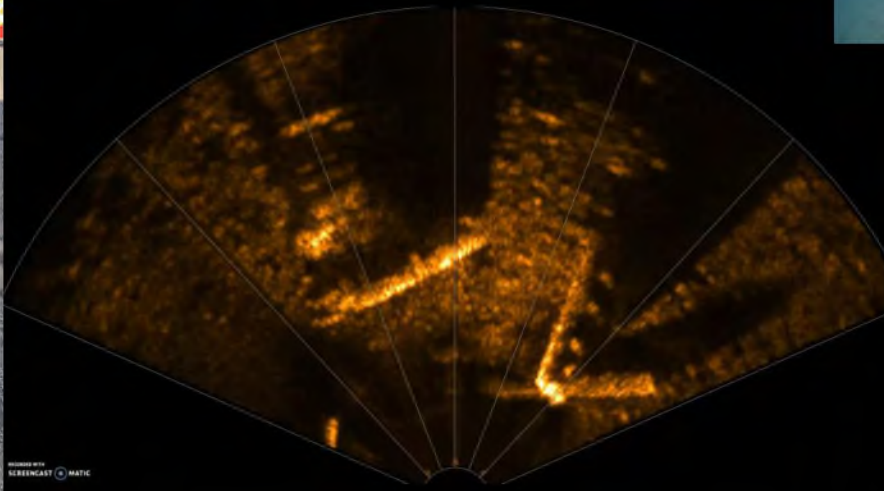
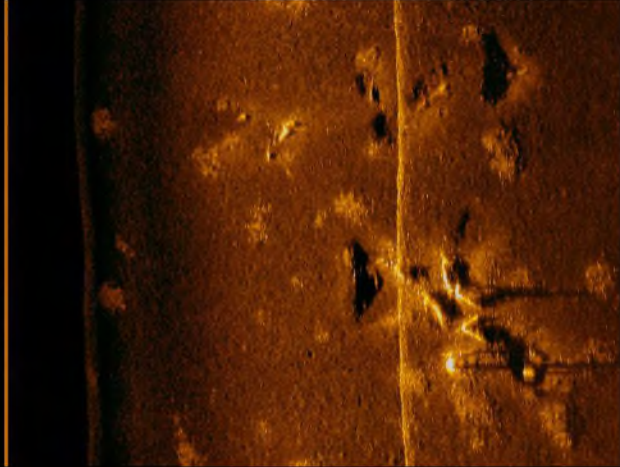
**FIX**

Dive Navigator



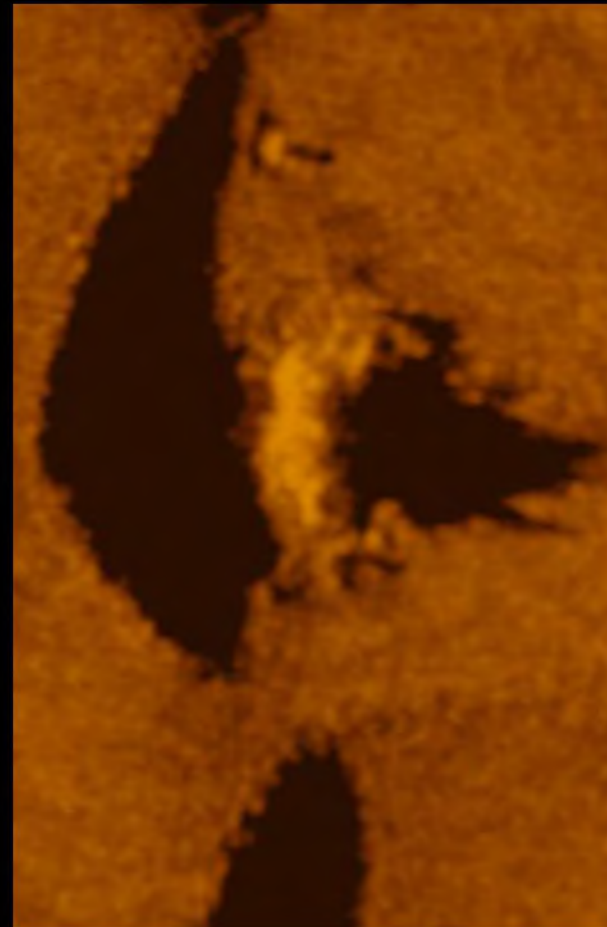
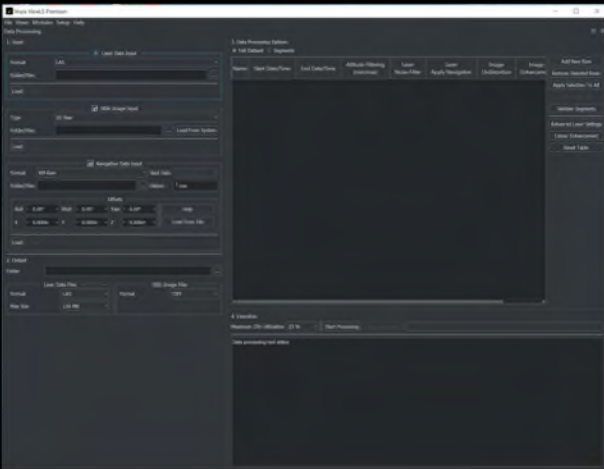
# FIX

Remotely Operated Vehicle





# VOYIS



Wide-Area Search

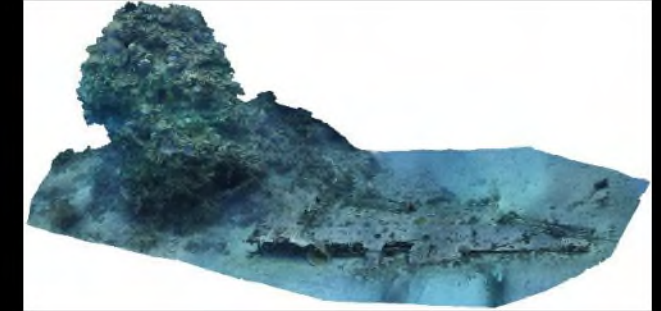
# Find

Target Reacquire

# Fix

Target Documentation

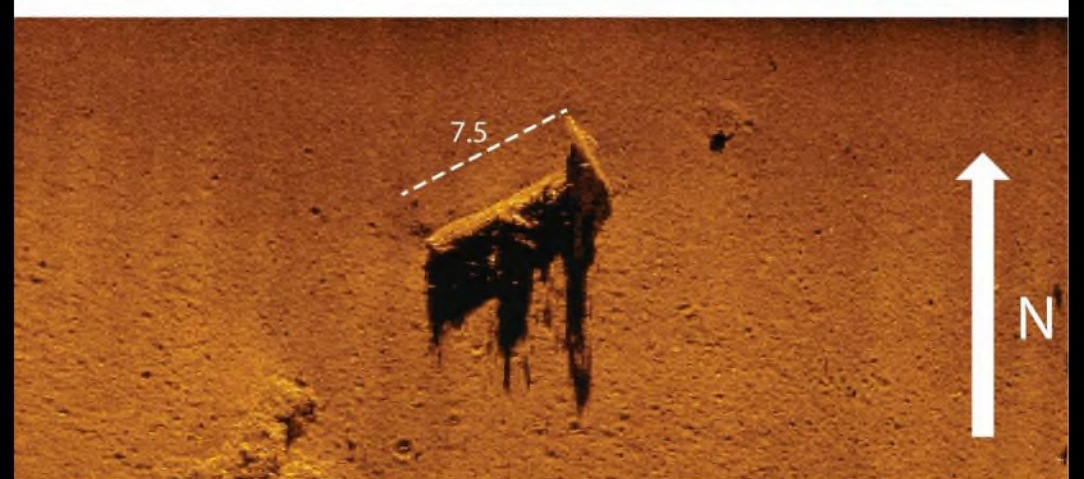
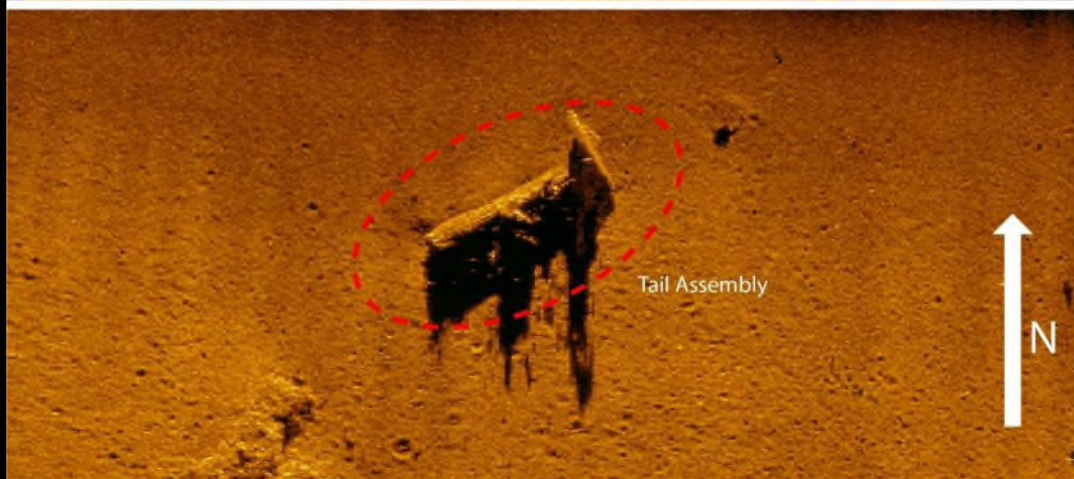
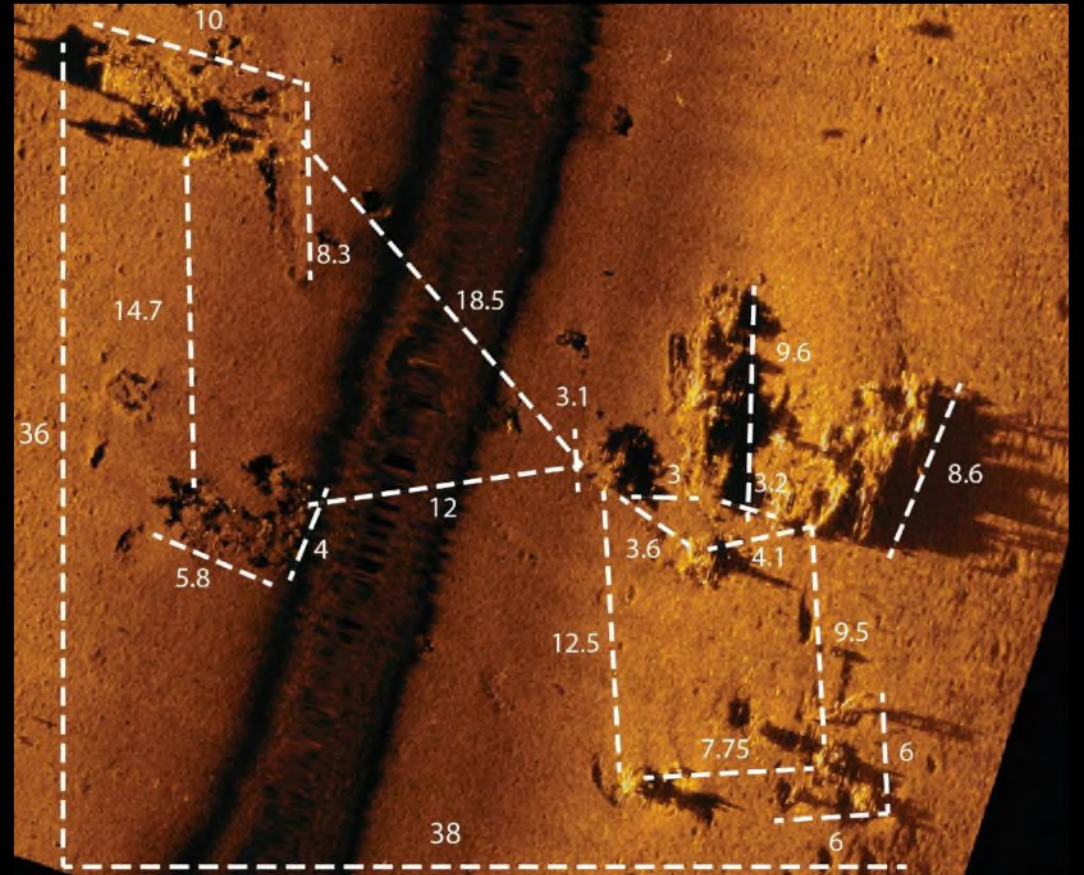
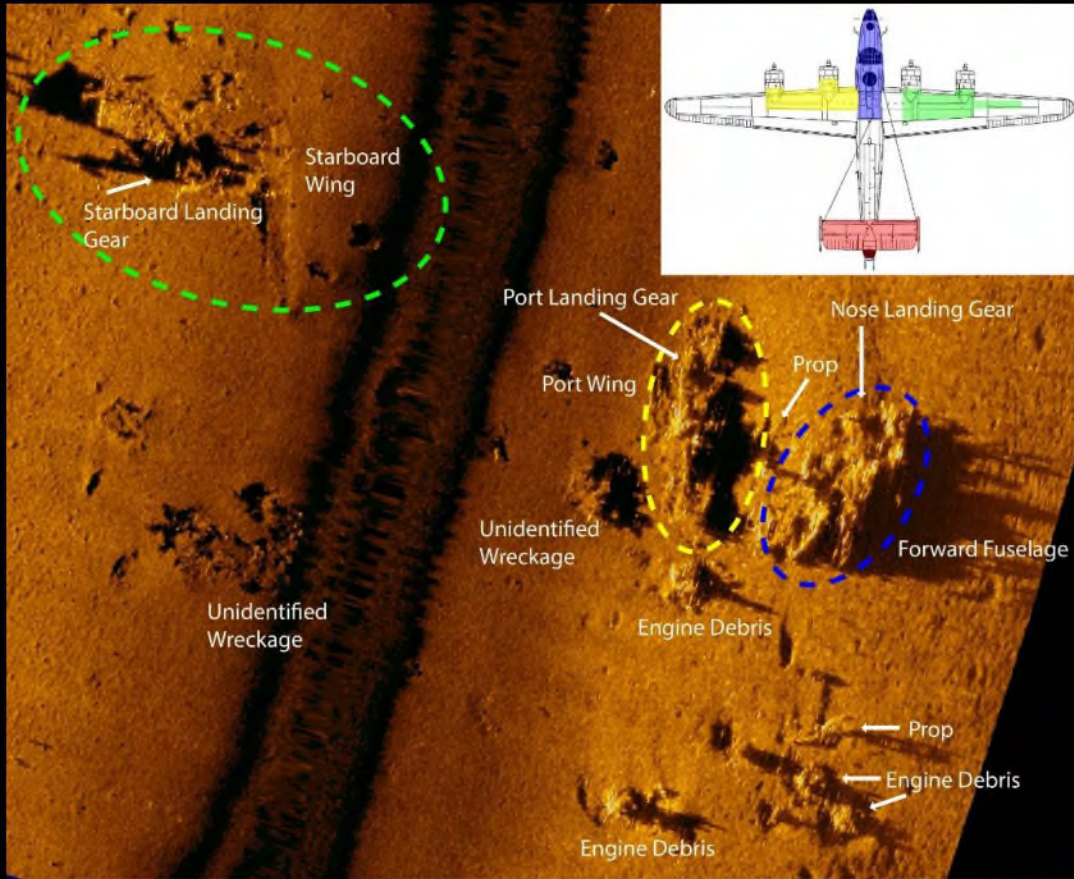
# Finish



Sensor	FIND	FIX	FINISH
UUV REMUS 100 sidescan sonar 600 kHz	✓	•	•
Vessel-based multibeam (T50)	✓		•
UUV REMUS 100 magnetometer	✓	✓	•
UUV REMUS 100 multibeam		✓	•
UUV REMUS 100 sidescan sonar 1200/1800 kHz	•	✓	✓
UUV REMUS 100 low light imager	•	✓	✓
Diver based navigator	•	✓	✓•
Barracuda ROV	•	✓	✓
Diver based digital camera	•		✓

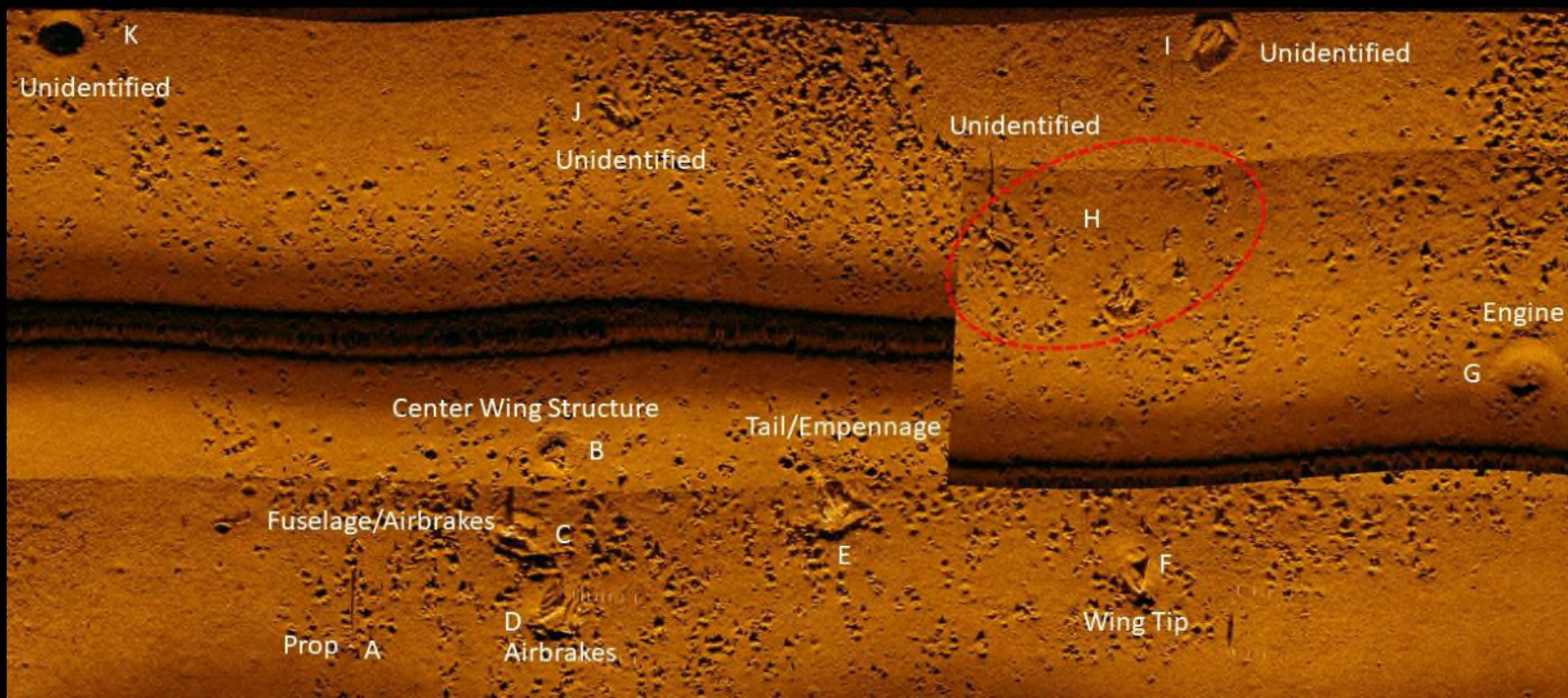
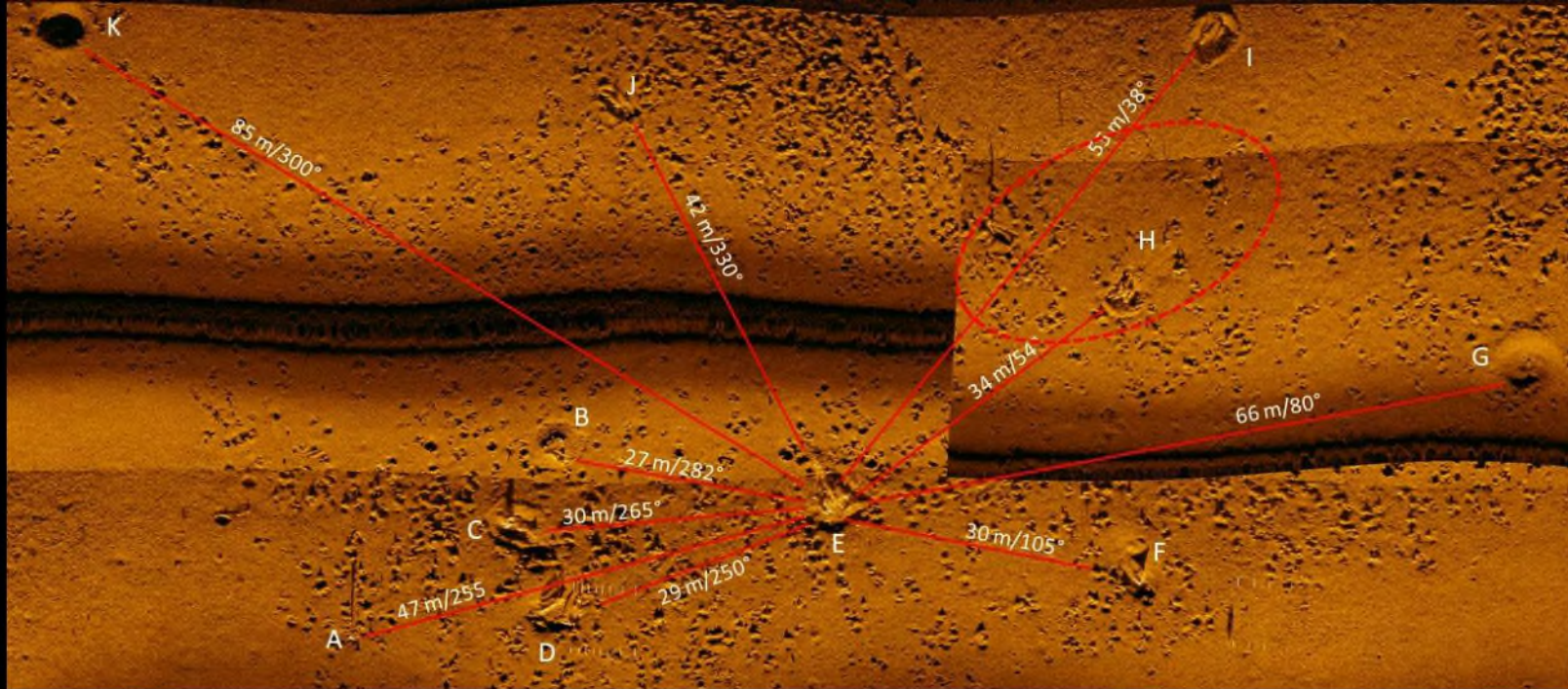
Site Condition	UUV - SSS - Visible	ROV - Imaging Sonar - Visible	Multibeam Sonar	Sub Bottom Profiler	Diver - Imaging Sonar - Visible
Rocky low slope	✓✓✓	✓✓	✓✓✓	X	✓
Rocky high slope	✓	✓✓	✓✓	X	X
Sand	✓✓✓	✓✓	✓✓✓	✓	✓✓
Mud Buried or Partially	✓✓	✓✓	✓	✓✓✓	✓
Coral Reef low slope	✓✓	✓✓	✓✓✓	X	✓✓
Coral Reef high slope	✓	✓✓	✓✓	X	X
High Flow	✓	✓	✓✓✓	✓	X
Low Visibility	✓✓	✓✓	✓✓✓	✓✓✓	✓
>50 m	✓✓✓	✓✓✓	✓✓	✓✓	X

**FINISH**

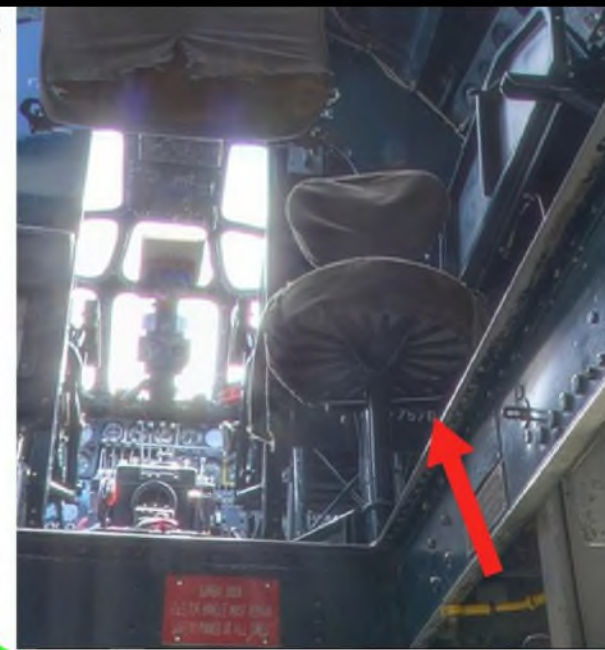
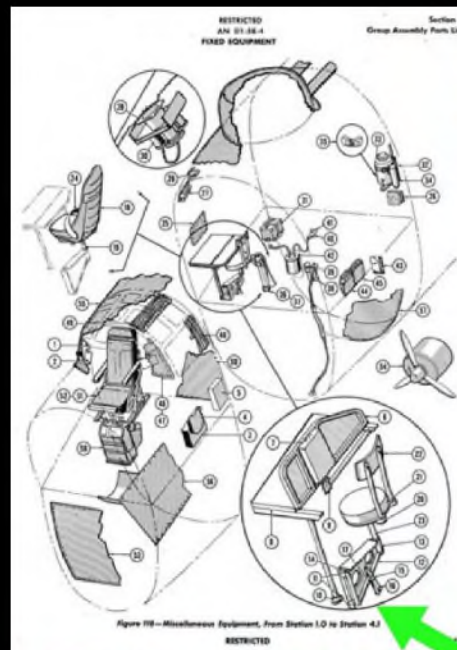


# FINISH

REMUS 100- 1200/1800 Khz

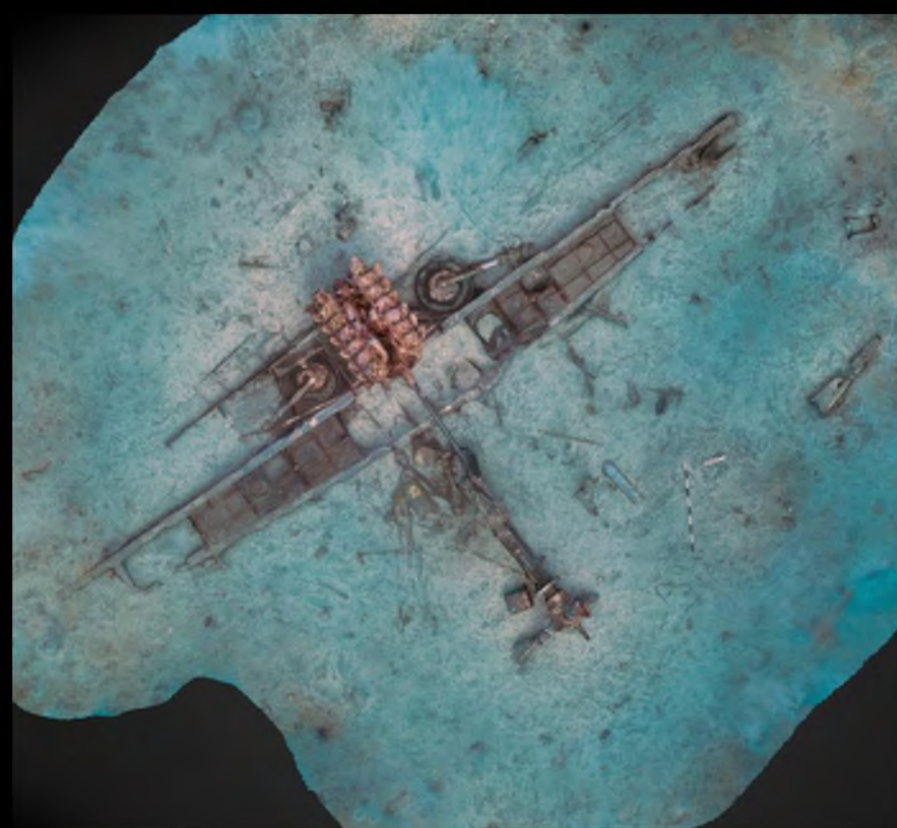


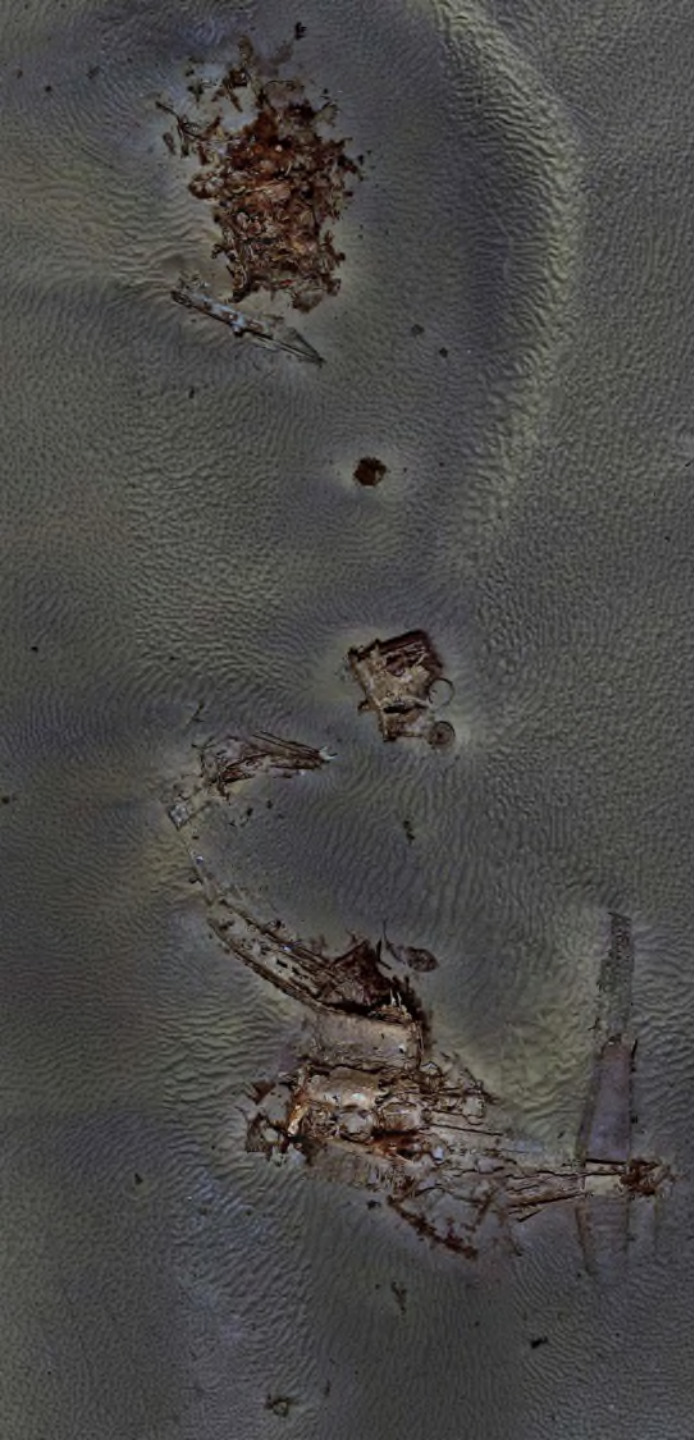
# FINISH ROV



# FINISH

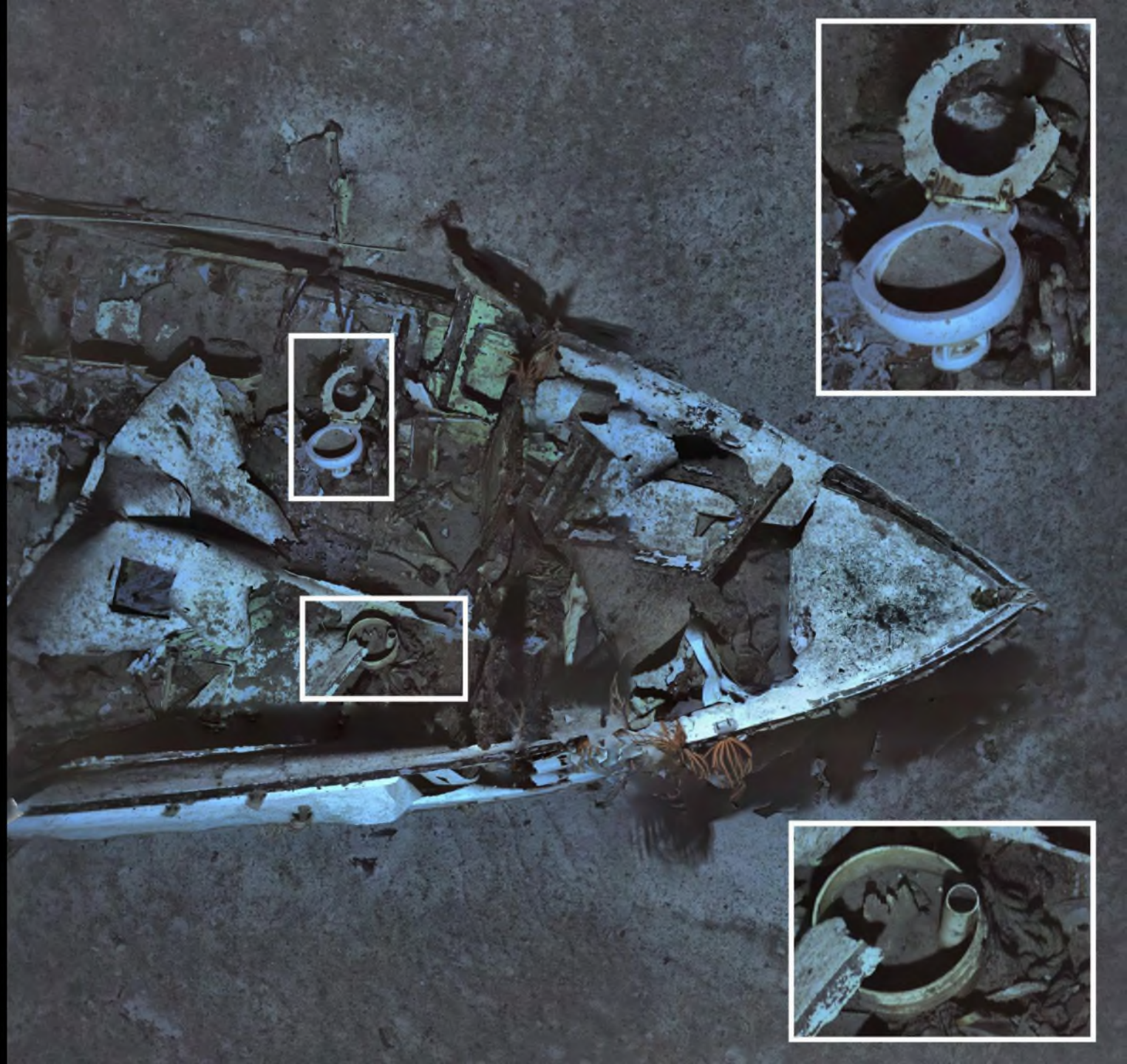
Diver-based Camera  
Photogrammetry



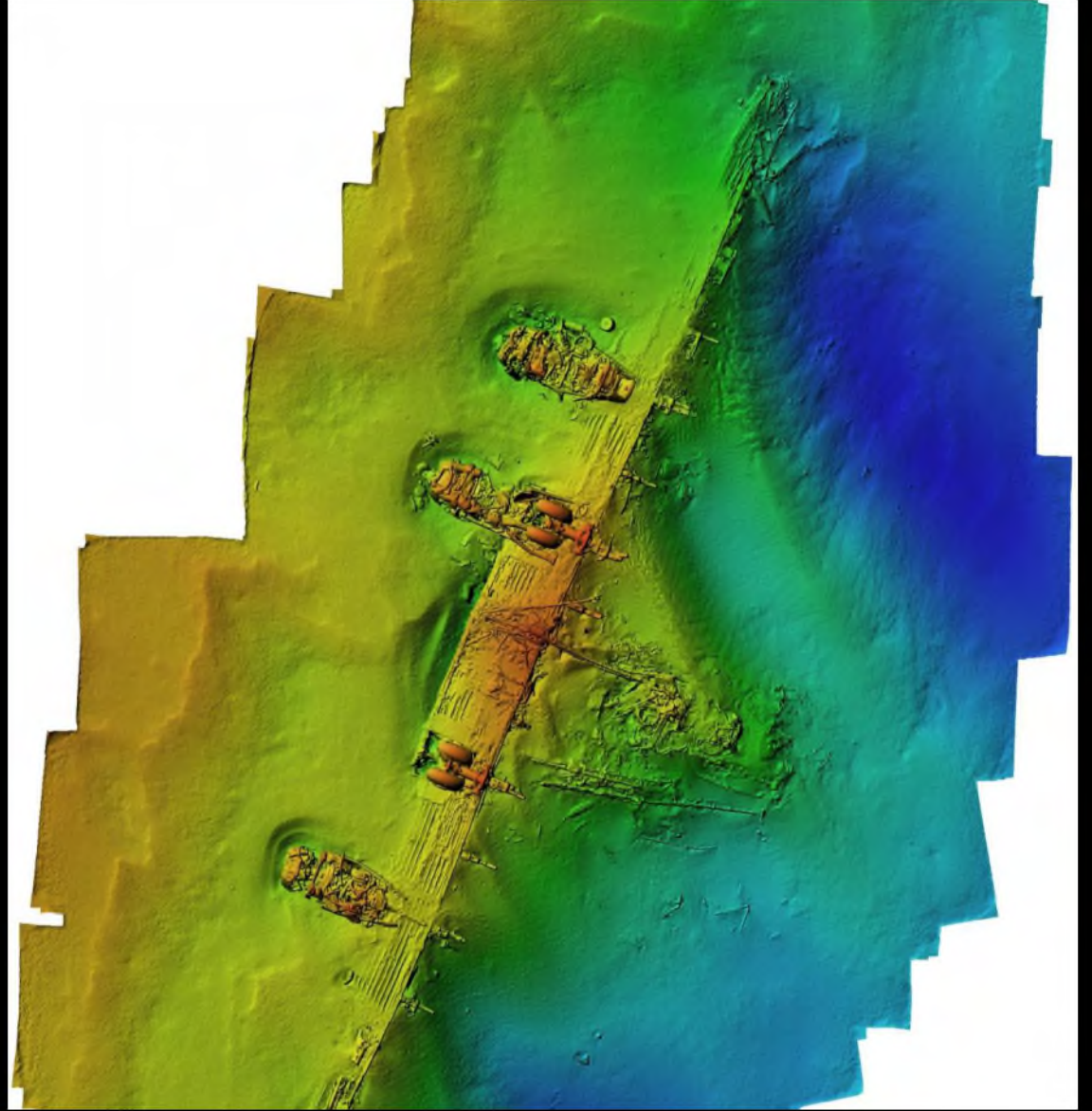


# FINISH

AUV-based Camera  
Photogrammetry









Orthomosaic, True Color



Digital Elevation Model





2023/2024



2022

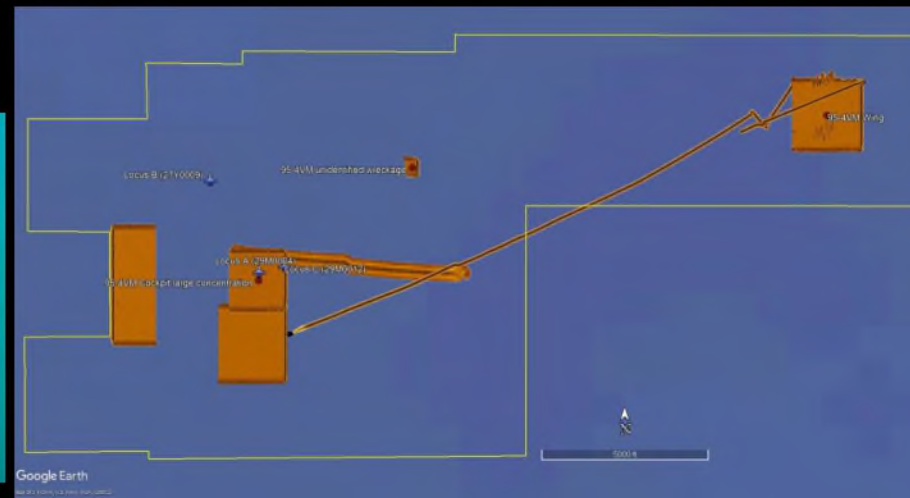
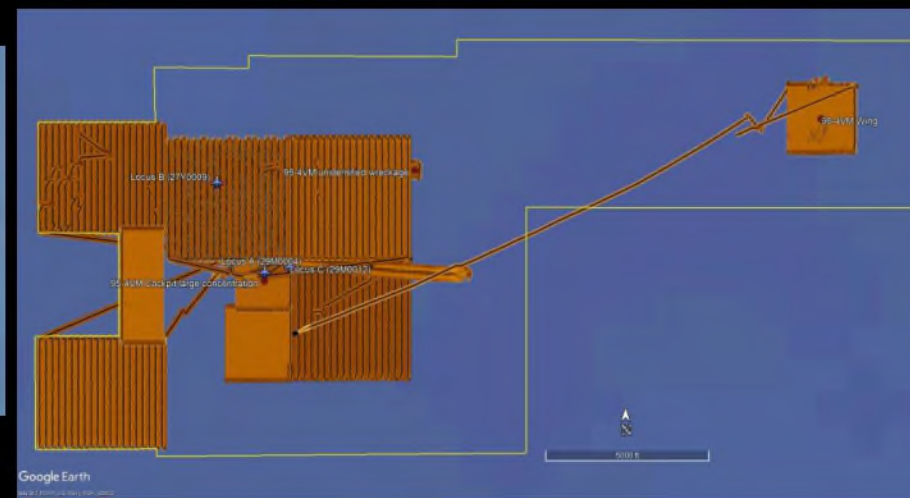


2020

Loss Date	Aircraft Type	REFNO	MIA	Survey year
7/7/1967	B-52 Stratofortress	0757/0758	4	2020
4/29/1975	CH-47D Sea Knight	2042	2	2022
9/18/1965	A-6A Intruder	0148	2	2023
1/22/1966	S-2D Tracker	0237	4	2023

REFNO 0757/0758

**Case Profile: Mid-air Collision of two Guam-based B-52 Aircraft (call signs "Kilo Red 1" and "Kilo Red 2") on the way to their bombing target in South Vietnam. Extensive search efforts recovered 7 survivors from both aircraft. The site of Kilo Red 1 was discovered in 1990 by VN divers, relocated by USN divers in 1994, but has since been lost. At the time of the survey, 1 MIA was still associated with Kilo Red 1, and 3 MIA were associated with Kilo Red 2.**





**Case Profile: Crashed into the water during a nighttime landing approach to the USS Hancock. The helicopter was providing SAR support during the evacuation of Saigon. Two MIA remain associated with this loss incident.**



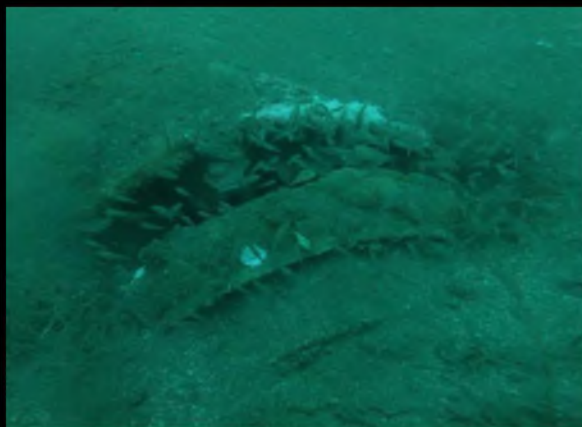
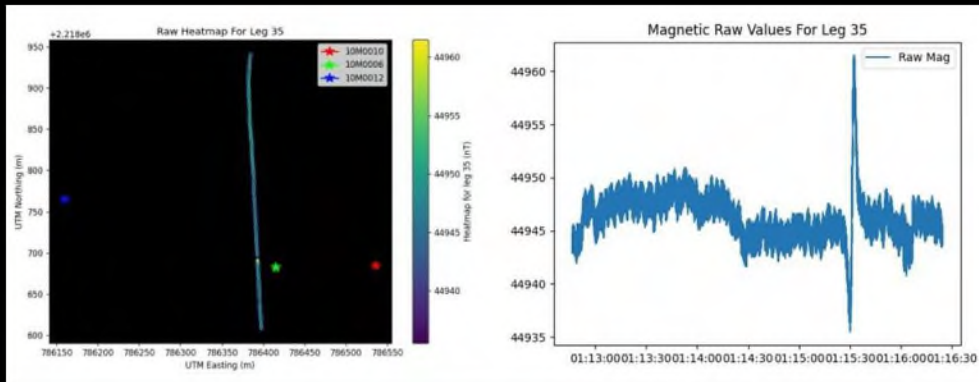
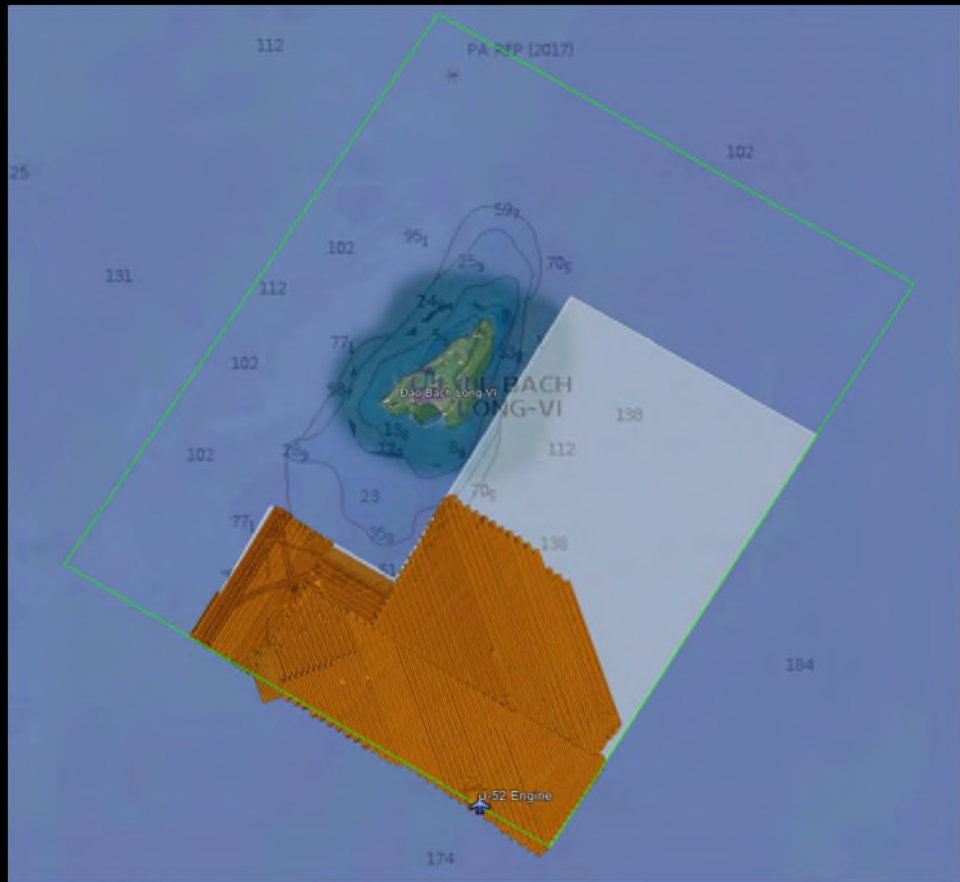
12 days  
 3 REMUS 100 AUV  
 600 kHz SSS: 73 km<sup>2</sup>  
 1200 kHz SSS: 0.5 km<sup>2</sup>  
 Magnetometer: 0.5 km<sup>2</sup>  
 Results: No site  
 discovered

# REFNO 0148/0237 (2023)

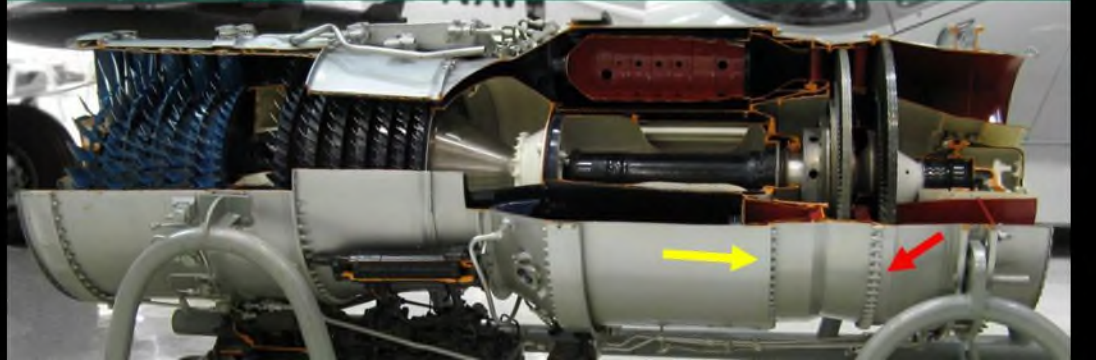
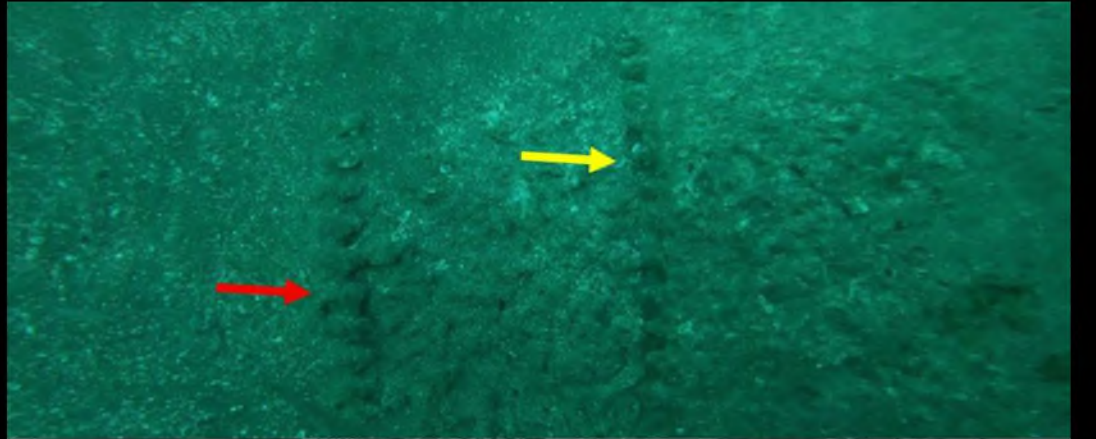
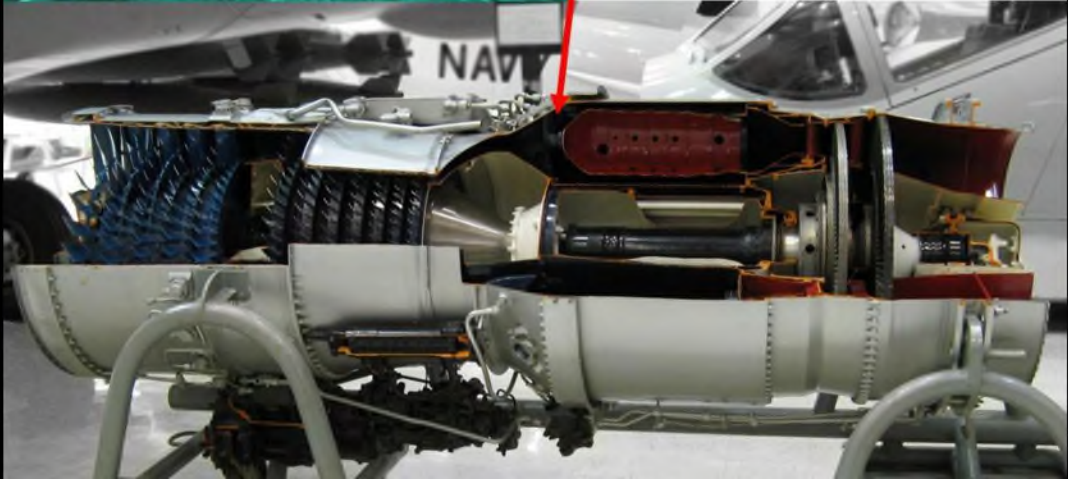
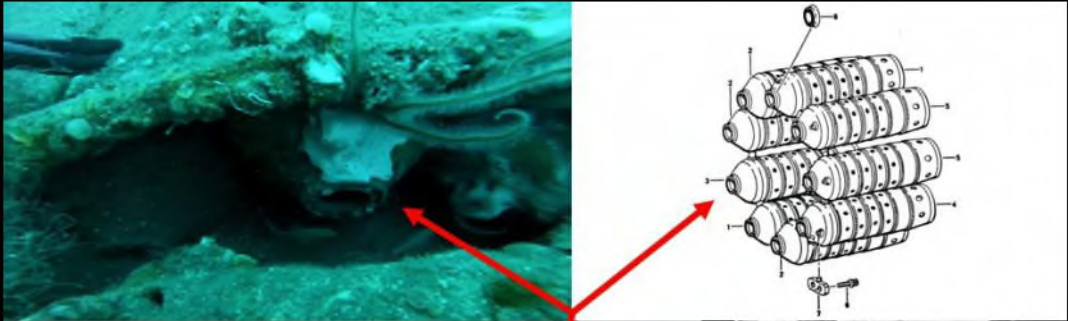
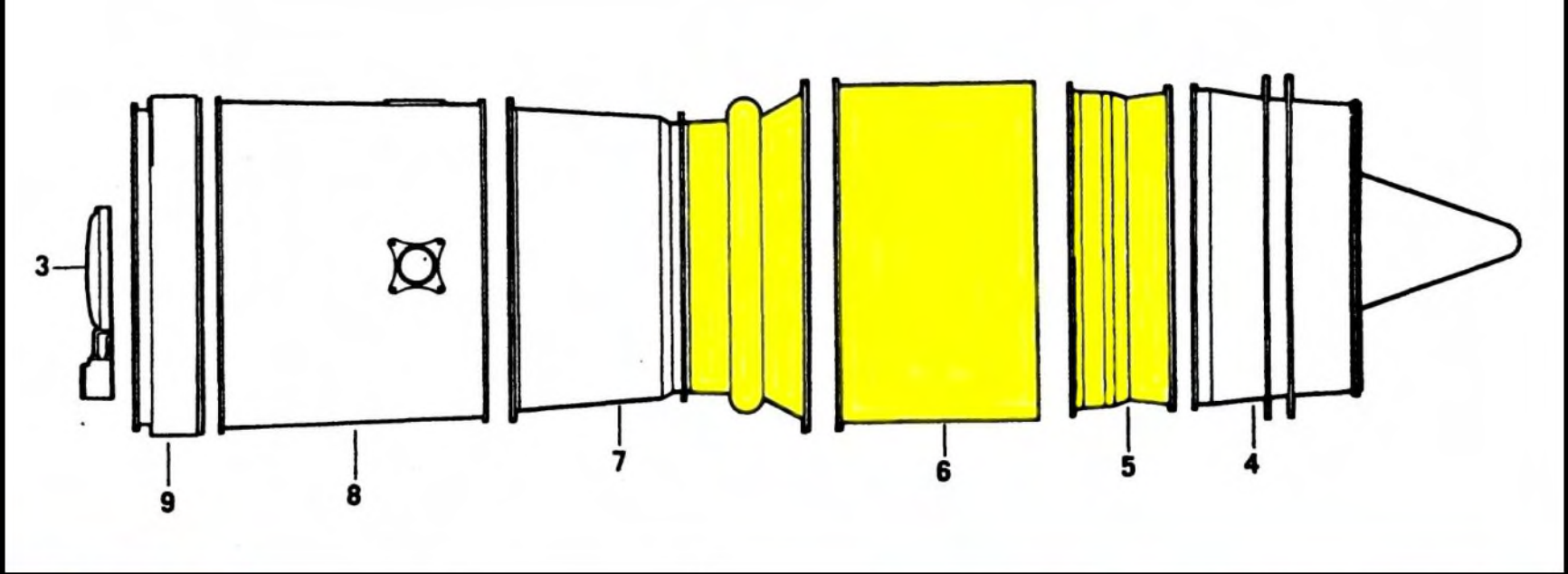
**Case Profile:** REFNO 0148 involves the loss of an A-6A intruder launched from the USS *Independence* aircraft carrier for a night-bombing attack against enemy patrol boats in the Gulf of Tonkin. The aircraft encountered anti-aircraft fire, and flight members observed the aircraft crash into the water and explode. The following day, after an extensive search for the two crewmembers, U.S. forces found only an oil slick. Two MIA are associated with this loss.

REFNO 0237 involves the over-water loss of an S-2D aircraft while on a surveillance mission, resulting in four unaccounted-for individuals.

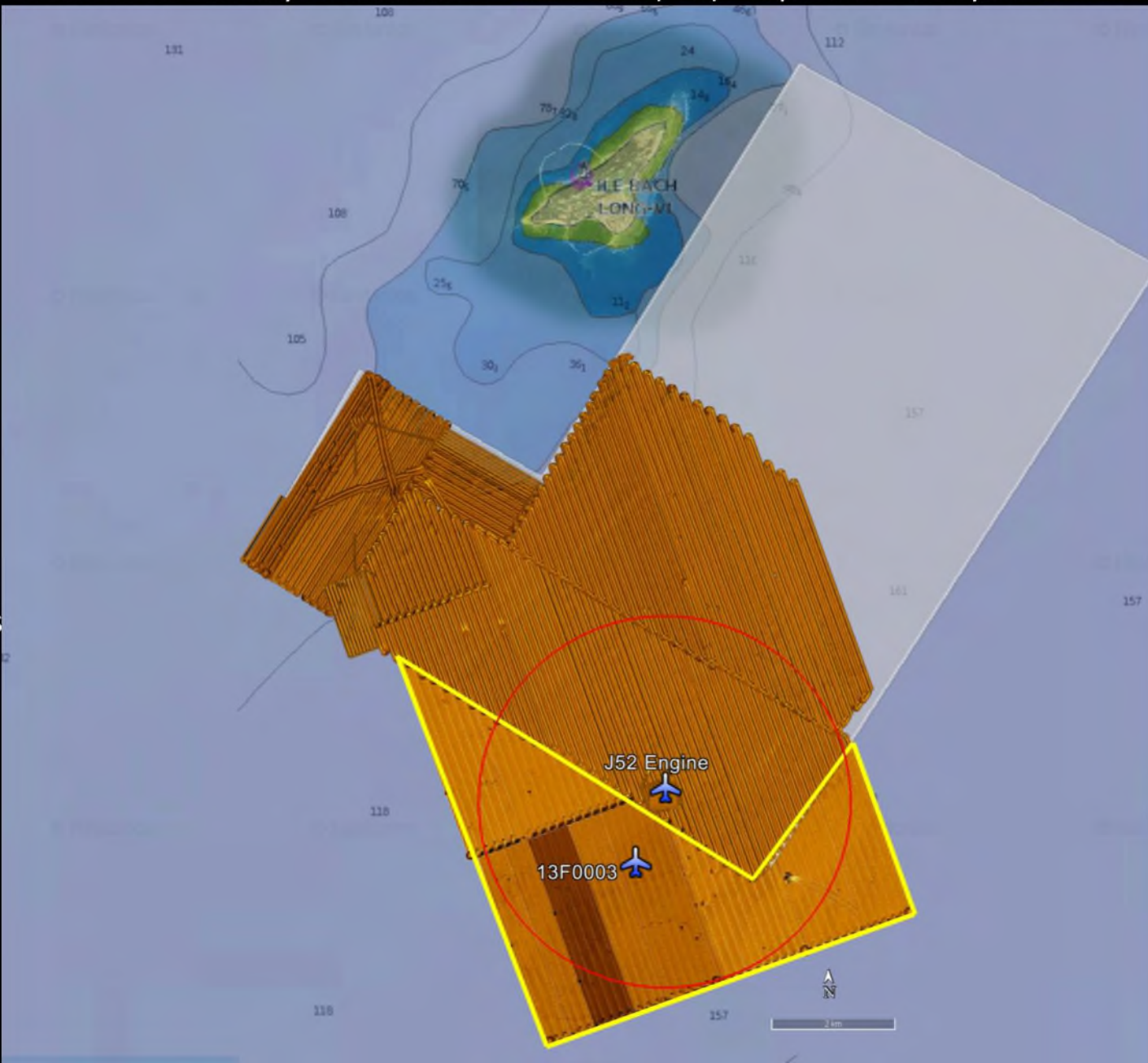
Vietnamese witnesses on Bach Long Vi Island gave details of two shootdowns of U.S. aircraft possibly associated with REFNO 0148 and 0237 based on dates, general location, and circumstances of loss.



12 days  
3 REMUS 100 AUV  
1 ROV  
600 kHz SSS: 48 km<sup>2</sup>  
1200 kHz SSS: 0.25 km<sup>2</sup>  
Magnetometer: 0.25 km<sup>2</sup>  
Results: Discovered 1 aircraft site



The continued survey focused on REFNO 0148 (A-6). Expanded survey to include a 3 km search radius centered on the J-52 engine found in 2023.





SIO was the first and remains DPAA's only non-governmental partnership conducting underwater searches in Vietnam



### Summary

MIA Survey Years: 2020, 2022, 2023, 2024

Total area surveyed: > 172 km<sup>2</sup>

Sites found: 2 (REFNO 0757 and REFNO 0148)



The remains of U.S.A.F Maj Paul Avolese were discovered and recovered by SIO during the 2020 survey. He was officially accounted for by DPAA on 21 Sept 2020 and laid to rest by his family.





DEFENSE POW/MIA ACCOUNTING AGENCY

# Scene Setter

**Mr. Kelly McKeague**  
**Director, DPAA**

*Vietnam War Annual Government Briefing 26-27 June 2025*



DEFENSE POW/MIA ACCOUNTING AGENCY

# Assisting in Your Own Case's Progress: A Family Perspective

Mrs. Karoni Forrester

Ms. Colleen Shine

Mr. Bruce Hallberg

Ms. Helen Hagerty

Facilitator: Dr. Franklin Damann, Director Nebraska Laboratory, DPAA

*Vietnam War Annual Government Briefing 26-27 June 2025*



DEFENSE POW/MIA ACCOUNTING AGENCY

# Other Resources for the Families

**Prof. Joseph Mortati**

**Mr. Glenn Hines, Australian Army/Navy (Ret.)**

**Colonel Ed Skyes, USAF (Ret.)**

**Facilitator: Dr. Jesse Stephen, Chief of Innovation, Partnerships and Innovation, DPAA**

*Vietnam War Annual Government Briefing 26-27 June 2025*











# Vietnam War AGB Afternoon Agenda

DEFENSE POW/MIA ACCOUNTING AGENCY



- |              |   |                               |
|--------------|---|-------------------------------|
| 12:15 – 1:15 | Lunch – On your own, in and around hotel                            |                               |
| 1:15 – 1:25  | SalesForce Taskers: What You Should Know                            | Mr. Rob Goeke, DPAA           |
| 1:25 – 1:40  | Scientific Challenges of Locating Identifiable Remains              | Dr. Debra Zinni, DPAA         |
| 1:40 – 2:00  | Single Nucleotide Polymorphism Next Generation Sequencing (SNP-NGS) | Dr. Tim McMahon, AFMES        |
| 2:00 – 2:55  | Questions and Answer Session  | Mrs. Jennifer Nasarenko, DPAA |
| 2:55 – 3:00  | Closing Remarks   | Mr. Kelly McKeague, DPAA      |



**AGB Survey**



DEFENSE POW/MIA ACCOUNTING AGENCY

# SalesForce Taskers

Mr. Rob Goeke  
Outreach and Communications Directorate

*Vietnam War Annual Government Briefing 26-27 June 2025*



- Salesforce is commercial cloud-based software
- Taskers are questions/requests from families (each tasker average 3 questions)
- 65 Vietnam War Taskers (as of May 28)
  - Army – 11
  - Navy – 7
  - Air Force – 45
  - Marine Corps – 2
  - Dept of State – 1

# Examples of Questions

DEFENSE POW/MIA ACCOUNTING AGENCY



1. I would like all information on single-seat aircraft losses within xx miles of the crash site.
2. I would like information on US prisoners captured in the area to see if they mentioned seeing him. What was the PAVN process for handling POWs and isolating new POWs from other POWs?
3. Please provide me all imagery of the area from the dates immediately preceding the loss and the dates after as well as all photos taken during field investigations related to the loss.
4. His wingman reported that he lost him in the confusion of battle and heavy cloud cover. Was the wingman ever interviewed by DPAA or (DPMO or JPAC) to see if he had further details?
5. My last analyst in 2018 promised me that he would research failures of ejection seats to determine the possibility that an ejection sequence never occurred. Why was that never done?

# Examples of Questions

DEFENSE POW/MIA ACCOUNTING AGENCY



1. I found three documents from White House and CIA officials from 1973 that were not found in my loved one's file. Why were these files not incorporated into the case file?
2. I would like the SAR logs for the month of the loss to see if there is anything in there that was missed that might correlate to the loss.
3. How much of his aircraft was recovered by the Vietnamese in 1972 and where did they take the wreckage? Where is the wreckage today? I would like photos of the wreckage.
4. Will DPAA interview the descendants of the eyewitness or villagers to see if they have new information that could shed light on our case? Perhaps the witnesses were reluctant to share info in 1994.
5. Request DPAA read through the returnee debriefs of xxxx, xxxxx, xxxx, xxxxx, and xxxx to see if there was any information that was missed that might relate to the case. The personnel who originally reviewed these debriefings were not very good.

# Conclusion

DEFENSE POW/MIA ACCOUNTING AGENCY



1. Taskers often combine multiple complex questions.
2. If time is of the essence, limit questions that are:
  - Speculative: Why didn't the SAR team search this area? Why did the analyst 10 years ago say "xyz"?
  - Technical: What was the failure rate of the Martin Baker ejection seat?
  - Mission/Training-related: What type of ordnance? How many flight hours did he have with crewmate?
  - Research Projects: I would like the details and records of all similar losses in the area.
3. Analysts are doing their best to work complex questions while analyzing leads for teams in the field.



DEFENSE POW/MIA ACCOUNTING AGENCY

# **Scientific Challenges of Locating Identifiable Remains**

**Dr. Debra Prince Zinni, D-ABFA  
Hawaii Laboratory Director**

*Vietnam War Annual Government Briefing 26-27 June 2025*

# Taphonomy - Acid Soil

DEFENSE POW/MIA ACCOUNTING AGENCY



# Taphonomy – Termite Mound

DEFENSE POW/MIA ACCOUNTING AGENCY



# Taphonomy – Termite Damage

DEFENSE POW/MIA ACCOUNTING AGENCY

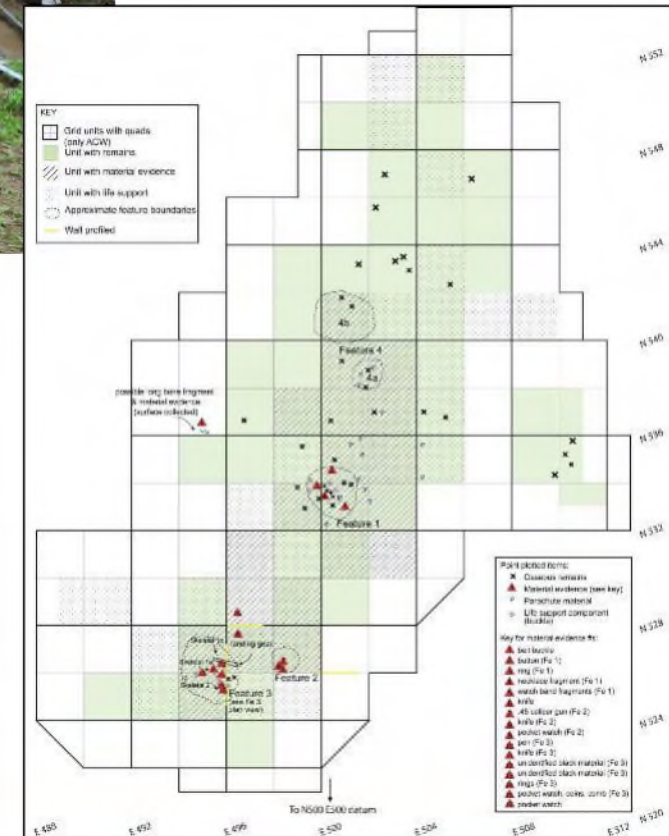


# B-24 Aircraft – Papua New Guinea

DEFENSE POW/MIA ACCOUNTING AGENCY



## 70 acres



# F4-C Fighter – Crashed at Low Angle Vietnam

DEFENSE POW/MIA ACCOUNTING AGENCY



- 1 excavation – 772 m<sup>2</sup>

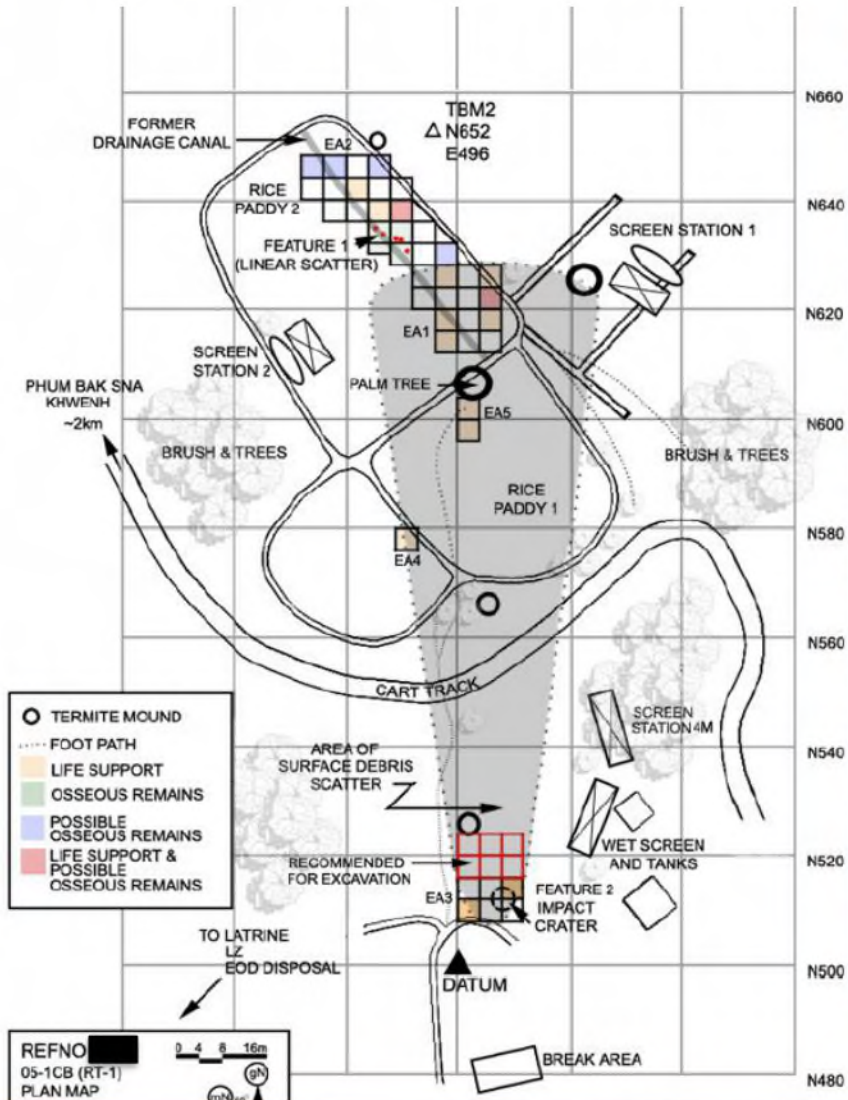


# F-100D Fighter – Fast moving aircraft Cambodia

DEFENSE POW/MIA ACCOUNTING AGENCY

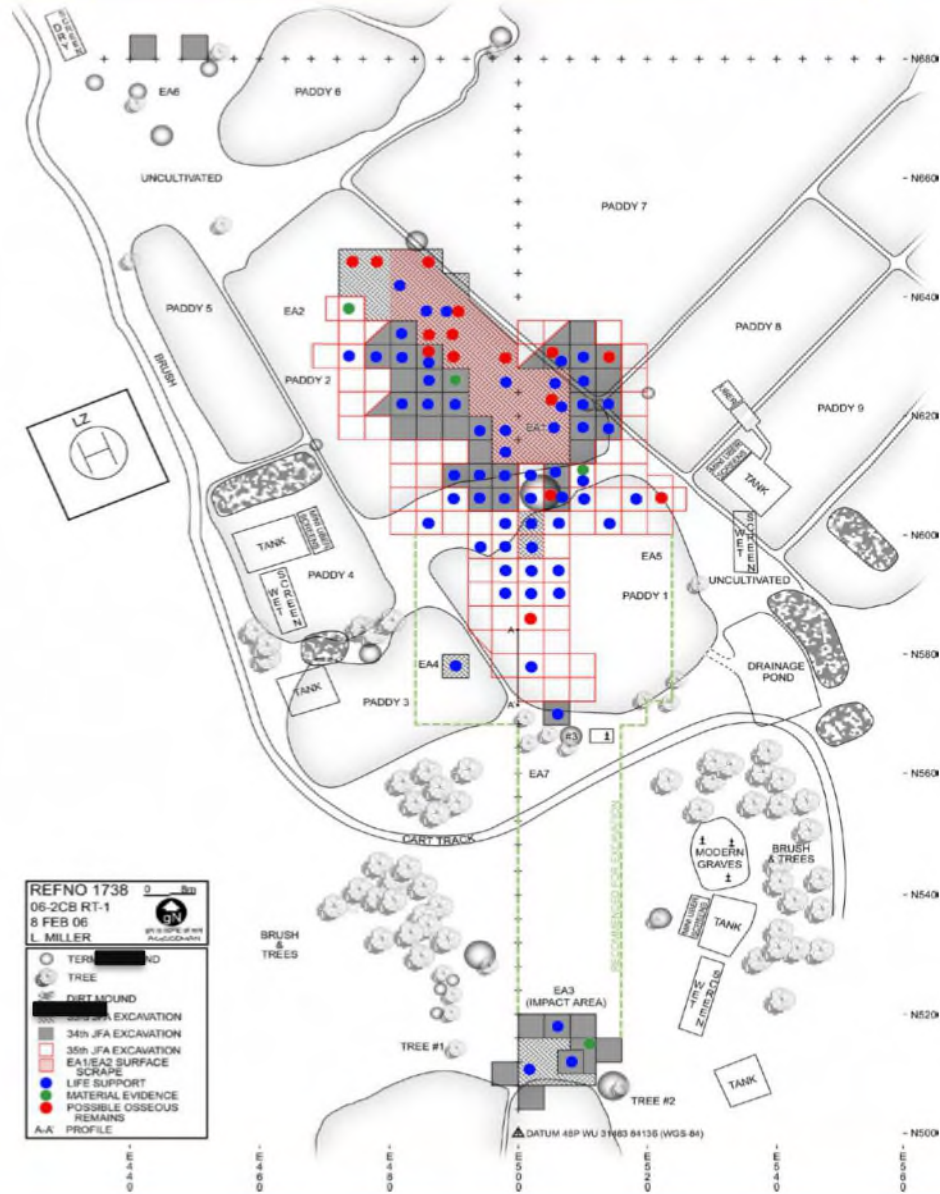


- 1 excavation – 3136 m<sup>2</sup>



# F-100D Fighter – Fast moving aircraft Cambodia

DEFENSE POW/MIA ACCOUNTING AGENCY

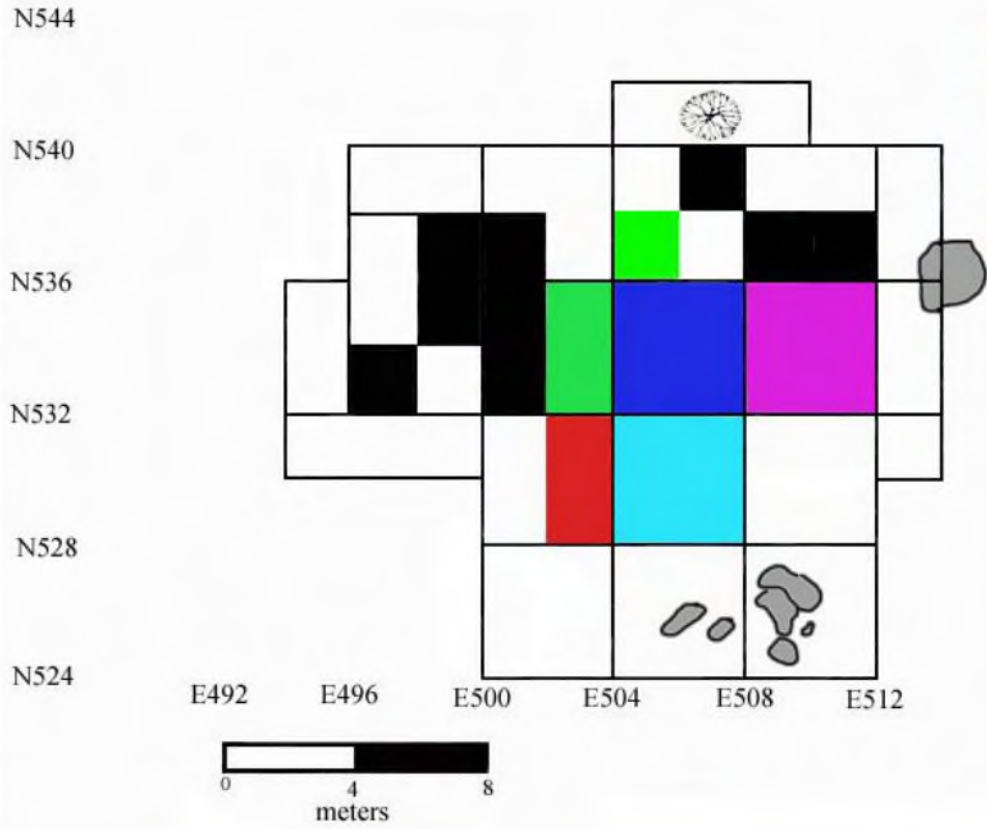


# UH-1H – Slow Moving Laos

DEFENSE POW/MIA ACCOUNTING AGENCY

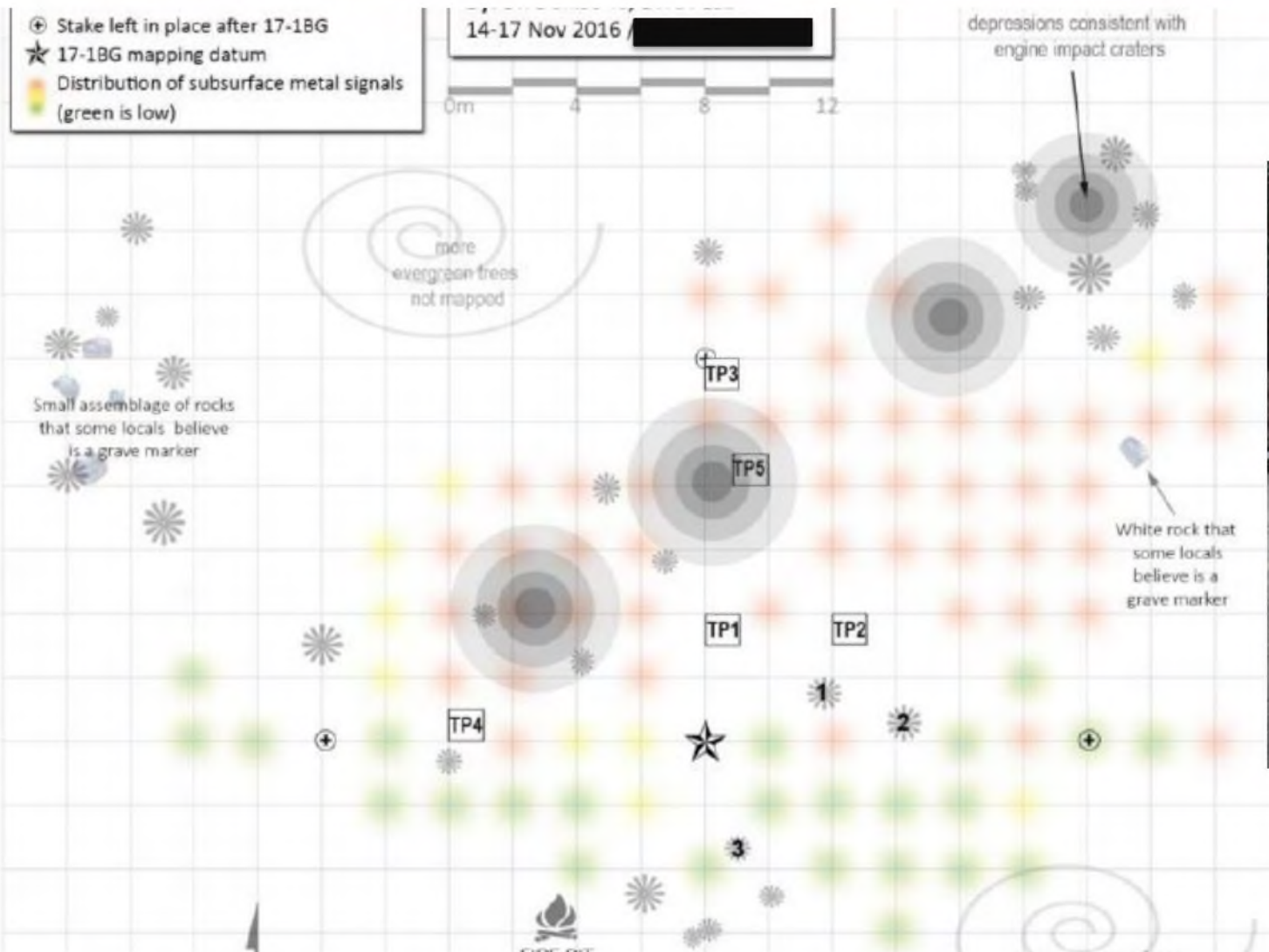


1 excavation – 276 m<sup>2</sup>  
16 cmbs



# B24H – Slow Aircraft Crash Bulgaria

DEFENSE POW/MIA ACCOUNTING AGENCY



# F-4 – Fast moving, High Angle Laos

DEFENSE POW/MIA ACCOUNTING AGENCY



2<sup>nd</sup> excavation  
376 m<sup>2</sup>  
17 to 370 cmbs



# F-4 – Fast moving, High Angle Laos

DEFENSE POW/MIA ACCOUNTING AGENCY



2 excavations – 696 m<sup>2</sup>

17 to 370 cmbs



# B-25C Mitchell Aircraft – Low Altitude Bombing Mission

DEFENSE POW/MIA ACCOUNTING AGENCY



# Comparison of Remains Recovered

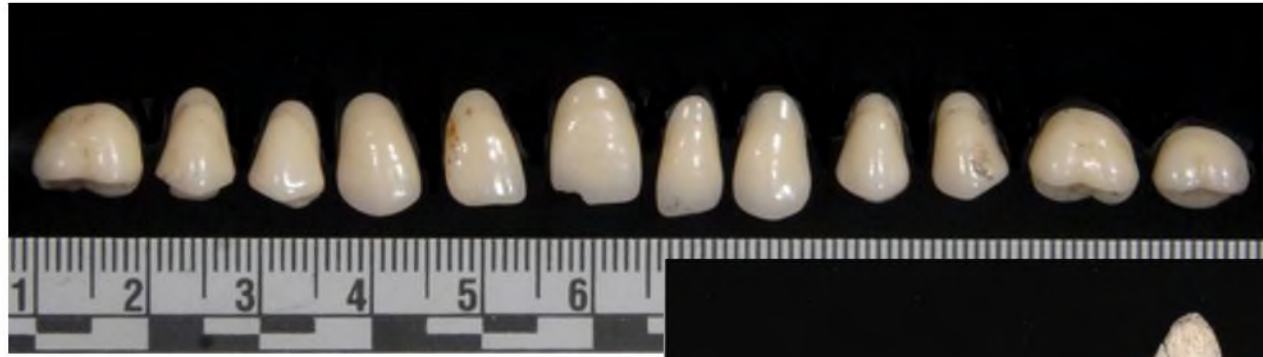
DEFENSE POW/MIA ACCOUNTING AGENCY



# UH-1H – Laos

DEFENSE POW/MIA ACCOUNTING AGENCY







- Aircraft fly faster
- Aircraft are larger
- During WWII, engines in fighter aircraft were in front of the pilot. By the Vietnam War, the engines shifted to behind the pilots
- Larger ordnance payloads
- New types of more powerful ordnance
- In some locations (primarily Laos), bombs were dropped on crash sites
- Metal scavenging
- Remains trade
- Taphonomic affects – Termites, Acidic soil, Vegetation regrowth in tropical environments
- Weather windows
- Development and landscape change from natural environments to cities
- Written records by other countries much scater when compared to WWII Europe
- Aging witnesses
- Host country constraints (e.g., PAX caps, Only having two base camps, two RTs per province)



# **DNA Analysis In The Identification Process**

**Timothy P McMahon, Ph.D.**

Director, DoD DNA Operations  
Armed Forces Medical Examiner System

Vietnam Annual Government Briefing  
June 26<sup>th</sup> and 27<sup>th</sup>, 2025



# Disclaimer



The opinions or assertions presented hereafter are the private views of the speaker(s) and should not be construed as official or as reflecting the views of the Department of Defense, its branches, the Defense Health Agency, the U.S. Army Medical Research and Materiel Command, the Armed Forces Medical Examiner System.



# Misconceptions on Government Capabilities



- By uploading your DNA profile to XXXX (if you haven't already), you contribute to a reference database that can encourage the U.S. government to use **cutting-edge Next Generation Sequencing technology** to identify previously unidentifiable remains.
- The Armed Forces DNA Identification Laboratory uses **limited sets of DNA tools** to match remains to family members.
- The Armed Forces DNA Identification Laboratory uses **antiquated DNA testing methods** to aid in the identification of missing service members



# Overview



- AFMES-AFDIL
- What AFMES-AFDIL brings to the table.
- New DNA testing Methods



# Armed Forces DNA Identification Laboratory (AFMES-AFDIL)



- The Armed Forces Medical Examiner System (AFMES)
  - Defense Health Agency (DHA)
- Established in 1990
  - Utilize DNA methods to identify the remains of US service members from Current Day and Past conflicts
- Accredited DNA Forensic Laboratory
  - ANSI National Accreditation Board (ANAB – ISO 17025 International Certification)
  - Federal Bureau of Investigation Quality Assurance Standards (FBI-QAS)
  - Accredited since 1998
- **Mission Partner with the Defense POW/MIA Accounting Agency (DPAA) since 1990**





# Notable Casework Advances



1992: First Use of MtDNA for Past Accounting Casework

1999: Use of Mini-Primer Sets

2006: **Demineralization Buffer**

2010: 12S rRNA: Human vs Non-Human

2013: Low Copy Number Y-STR testing

2014: Improved DNA Purification

2015: New 23 locus auSTR Kit

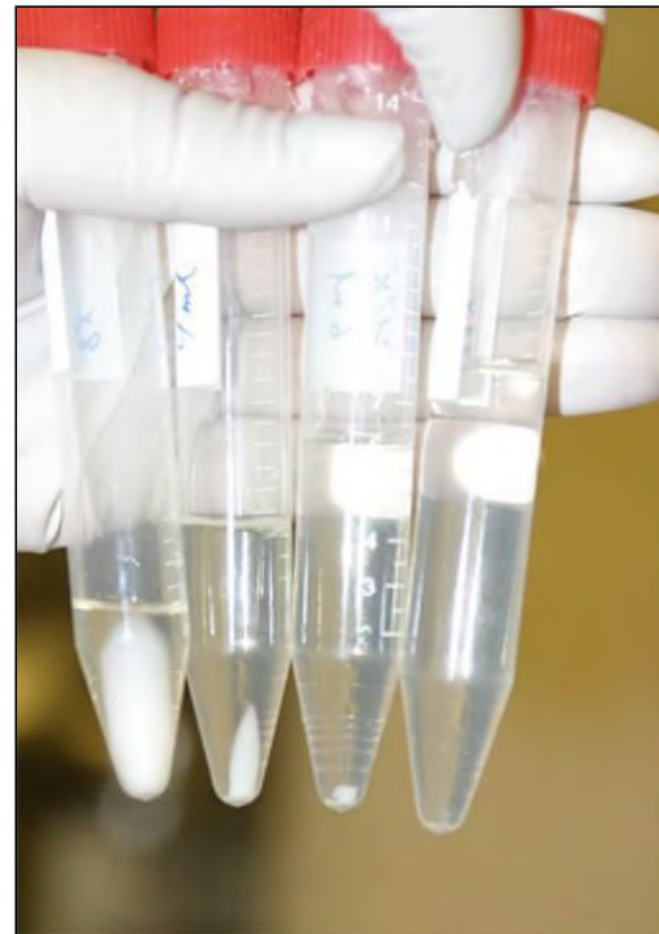
2016: **NGS mtDNA Capture Protocol for chemically modified samples**

2017: 15 New Scientists and additional NGS Team

2021: NextSeq validation

2025: **SNP Validation**

Future: Forensic Investigative Genealogy, DNA repair, removing non-human DNA???





# Why Does DNA Testing Take Time?

- Sample quality and DNA quantity
- Authentication
- Forensic crime
- Optimized Methods
- AFMES has to create new testing method
  - No commercial kits/have to develop
  - Science needs to catch up
- Family References

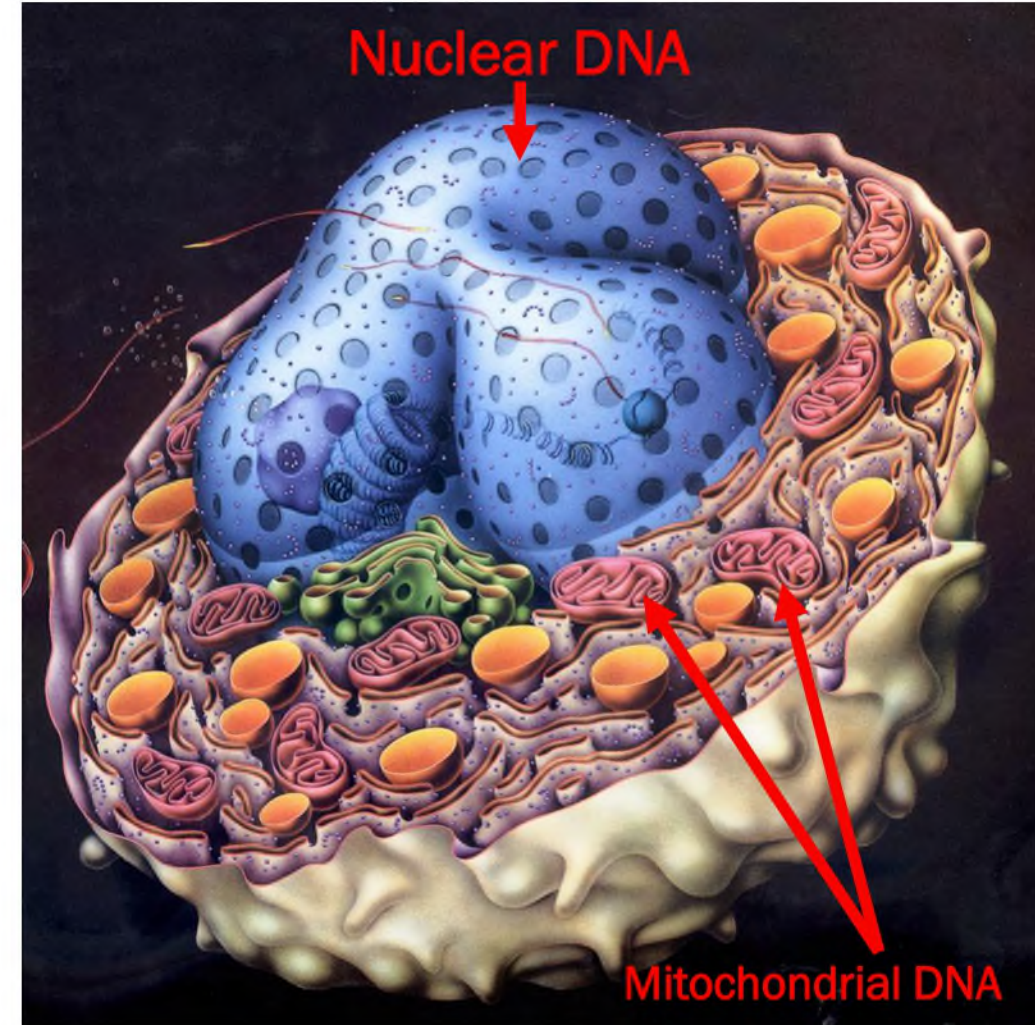
**Developing and implementing  
FIT FOR USE  
Methods**



# AFMES-AFDIL – DPAA Support



- Two Types of DNA in a cell
- Validated DNA testing methods:
  - 4 DNA extraction Methods
  - Sanger Mitochondrial DNA Sequencing
  - Autosomal STRs
  - Low copy Number Y Chromosomal STRs
  - Next Generation mitochondrial genome Sequencing
  - Next Generation Nuclear SNP/Kinship





# Multiple Testing Methods



- AFMES-AFDIL needs to be flexible and have many alternate DNA testing methods

## WHY?

- The DNA testing technology will be influenced by the submitted sample and what validated DNA testing methods a laboratory has on hand

## WHY?

- Remains comingled, fragmented, environmentally damaged and need to be able to work with samples submitted for testing.

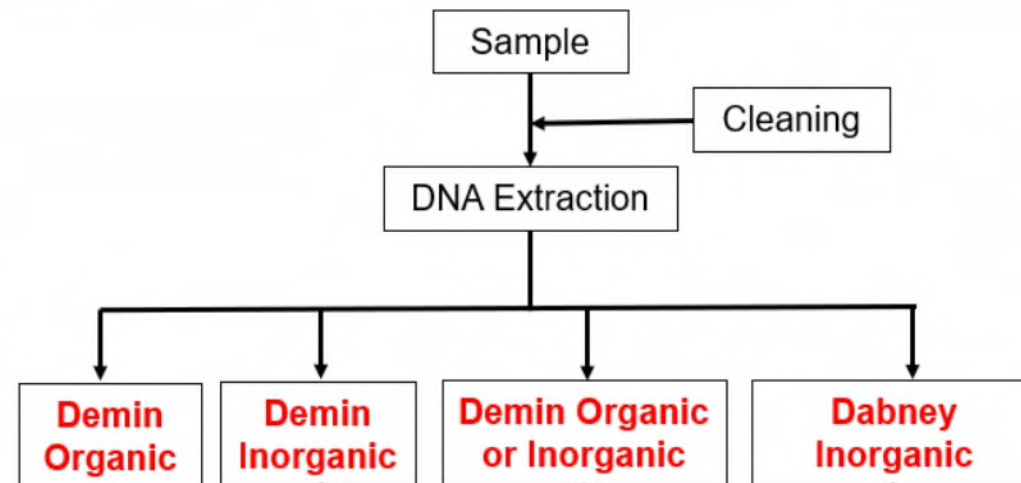
The more tools in your tool chest, the greater the chance of success



# Validated DNA Extraction Methods



- Typical Lab may have one or two extraction methods validated
- AFMES-AFDIL – 4 validated extraction methods



- Why 4 methods
  - High variation in sample types submitted by DPAA, allows ability to chose best extraction method for the sample condition
  - Ability to chose a **FIT For Use Extraction** method – Increase success rate



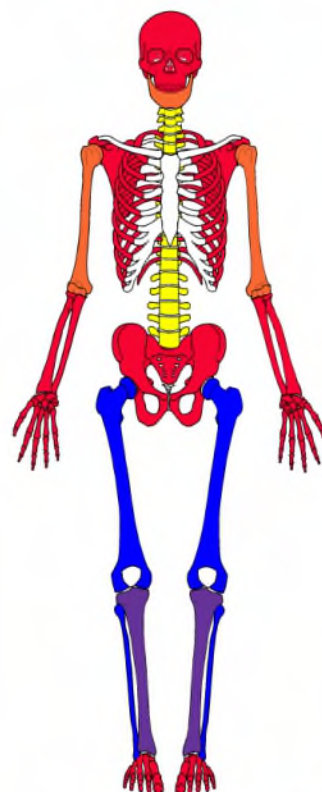
# Extraction Method Paired to DNA Testing

- Extraction method is based on DNA test:

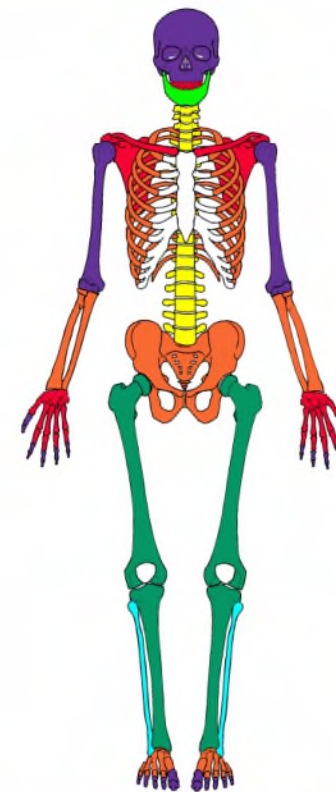


90-100
80-89.9
70-79.9
60-69.9
50-59.9
40-49.9
30-39.9
0-29.9

mtDNA Sanger Sequencing  
Organic Extraction



Autosomal STR (Minifiler)  
Organic Extraction



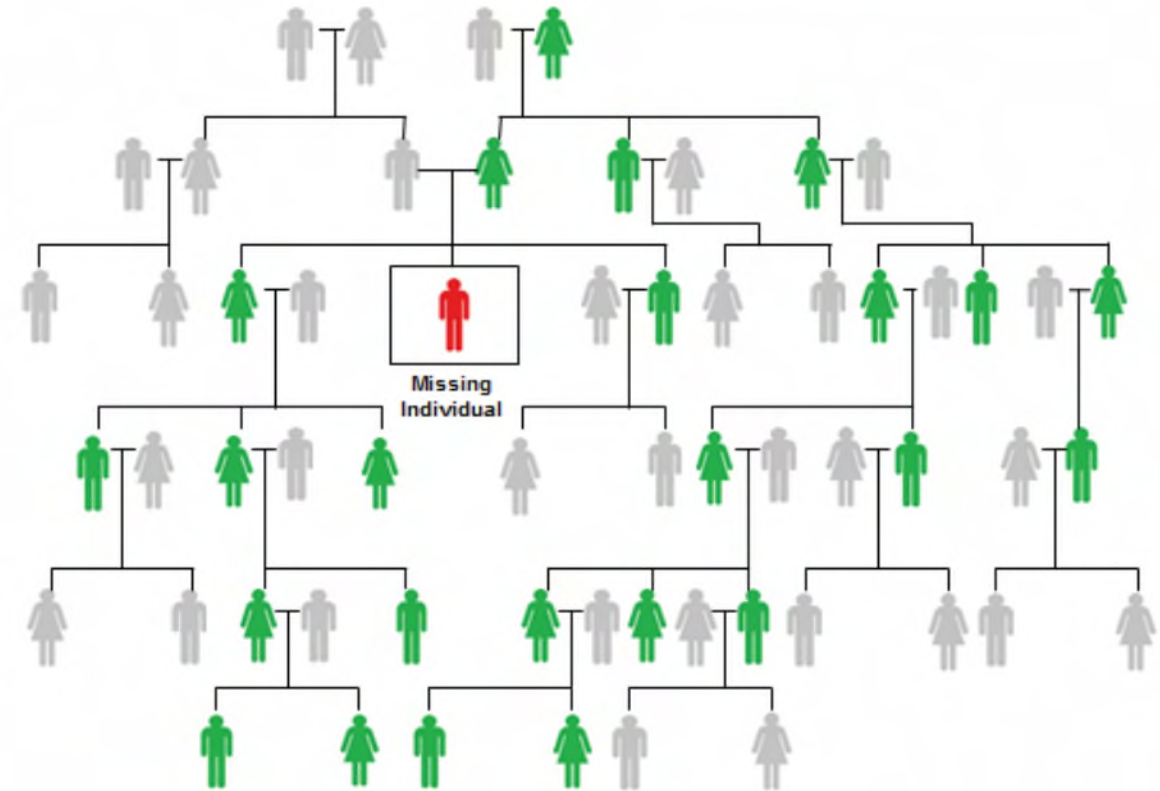
Autosomal STR (Minifiler)  
Inorganic Extraction



# Why mitochondrial DNA?



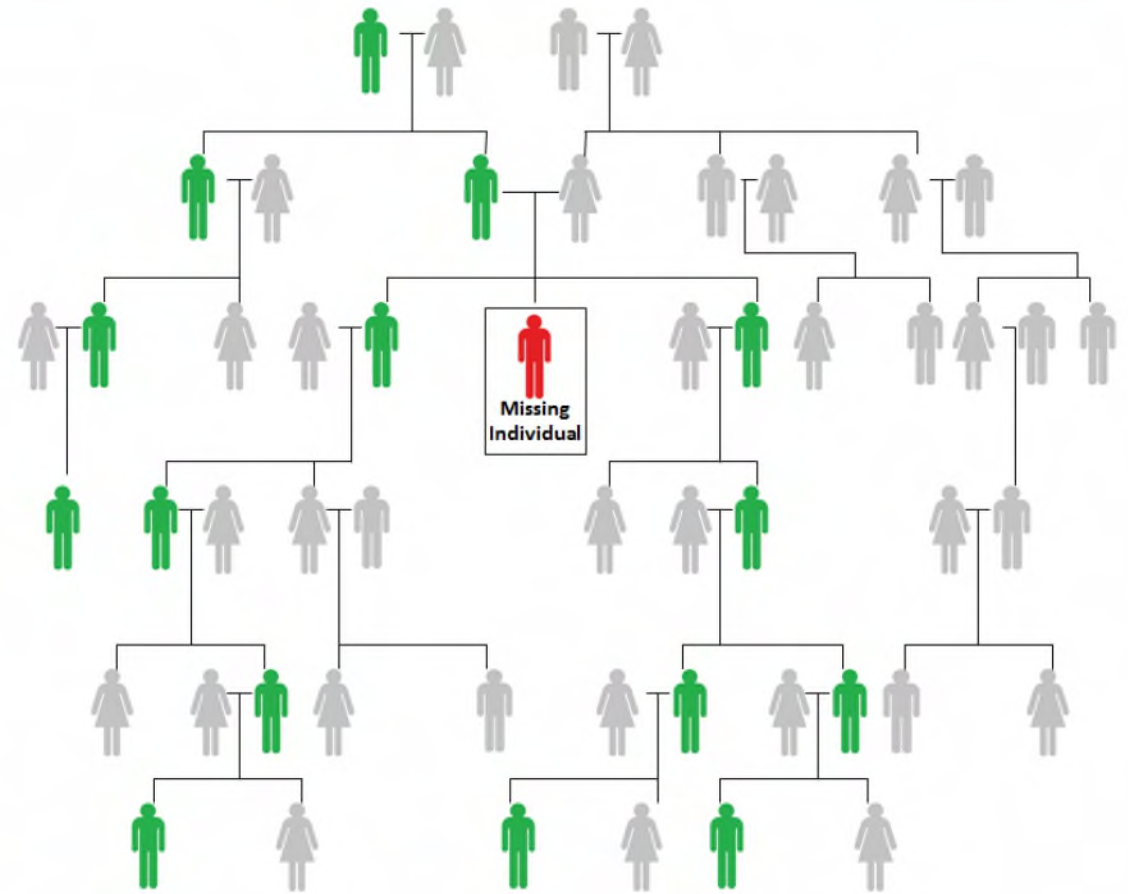
- mtDNA exists in thousands of copies in the cell
- DNA in older samples is often degraded
- Gives a greater depth of family reference materials
- Develop methods for highly degraded DNA
  - Great tool for sorting
  - Common and unique sequences
  - Groups remains and guides additional testing.





# Nuclear DNA: Y Chromosomal STR Testing

- Standard Commercial kit
  - modified for DPAA samples
  - Low Copy Method – Not used by other labs
- Requires appropriate references
  - paternal lineage marker
  - Excellent for sorting remains
  - Provides strong combine statistics.
  - Not great for degraded or chemically treated samples

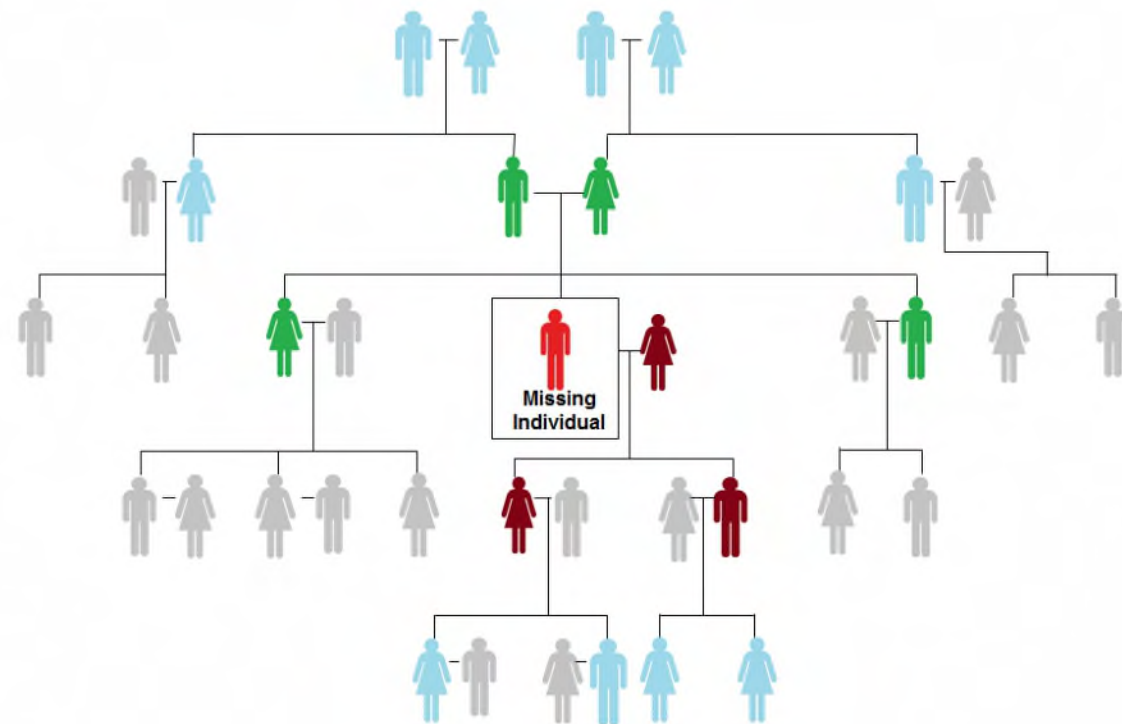




# Nuclear DNA: Autosomal STR Testing



- Standardized kit for crime work
  - Does not always work with degraded samples
  - Required no AFDIL modification to use the kit for DPAA samples
- Positive Identification when you have appropriate references
  - Mother, Father, Brother, Sister, Son, Daughter, Grand parents or Grandchildren





# AFMES-AFDIL Forensic DNA Testing Methods



	Mitochondrial DNA – Sanger Sequencing**	Nuclear DNA Low-Copy Number – YSTR*	Nuclear DNA Autosomal STR*	Mitochondrial DNA – Next Generation Sequencing***	Nuclear DNA Single Nucleotide Polymorphism (SNP)***
Fit for use?	Yes. Excellent for degraded DNA – non chemically treated	Maybe. Not great for degraded DNA.	Maybe. Not great for degraded DNA/chemically treated.		
Discrimination	Excellent sorting tool, <b>Maternal</b> lineage marker <b>good STR success</b>	Excellent sorting tool, <b>Paternal</b> lineage marker; good for combined statistic	Excellent! Positive form of identification with <b>appropriate Reference</b>		
Effort	LOTS of hands on. Completely manual.	Limited hands on but can be automated.	Hands on but can be automated		

\* Traditional test used in crime laboratories

\*\* Limited in Crime Laboratories

\*\*\* Only accredited laboratory with these methods



# AFMES-AFDIL Casework NGS Processing

2016

2016

2024

2025

Validated NGS Method for Mitogenome

R &D Capture Method for Nuclear SNPs

95,000 SNP Panel Validated

Casework Implementation (SNPs)

Hybridization capture enrichment

Optimization with multiple panels

Developmental Validation (QAS)

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsigen](http://www.elsevier.com/locate/fsigen)

ELSEVIER

Research paper

Performance evaluation of a mitogenome capture and Illumina sequencing protocol using non-probative, case-type skeletal samples: Implications for the use of a positive control in a next-generation sequencing procedure

Charla Marshall<sup>a,b,c</sup>, Kimberly Sturk-Andreaggi<sup>a,b</sup>, Jennifer Daniels-Higginbotham<sup>a,b</sup>, Robert Sean Oliver<sup>a,b</sup>, Suzanne Barritt-Ross<sup>a,b</sup>, Timothy P. McMahon<sup>a</sup>

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: [www.elsevier.com/locate/fsigen](http://www.elsevier.com/locate/fsigen)

ELSEVIER

Research paper

Extended kinship analysis of historical remains using SNP capture

Erin M. Gorden<sup>a,b</sup>, Ellen M. Greytak<sup>c</sup>, Kimberly Sturk-Andreaggi<sup>a,b,d</sup>, Janet Cady<sup>c</sup>, Timothy P. McMahon<sup>a</sup>, Steven Armentrout<sup>e</sup>, Charla Marshall<sup>a,b,c,e</sup>

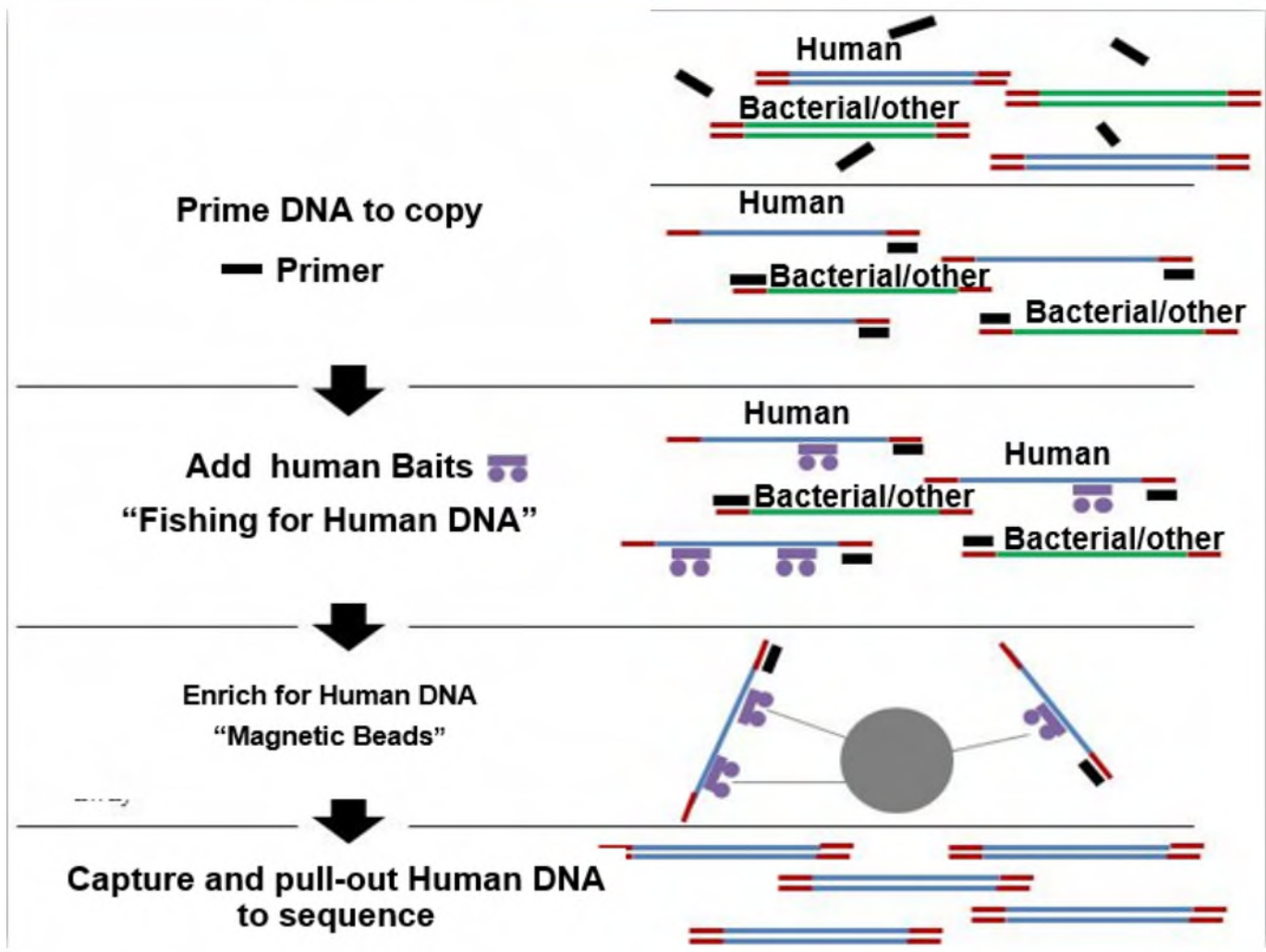
## 8. VALIDATION

STANDARD 8.1 The laboratory shall use validated methods for DNA analyses.

STANDARD 8.2 Developmental validation shall precede the implementation of any new methods used for forensic DNA analysis.



# Human mtDNA Whole Genome Capture Method

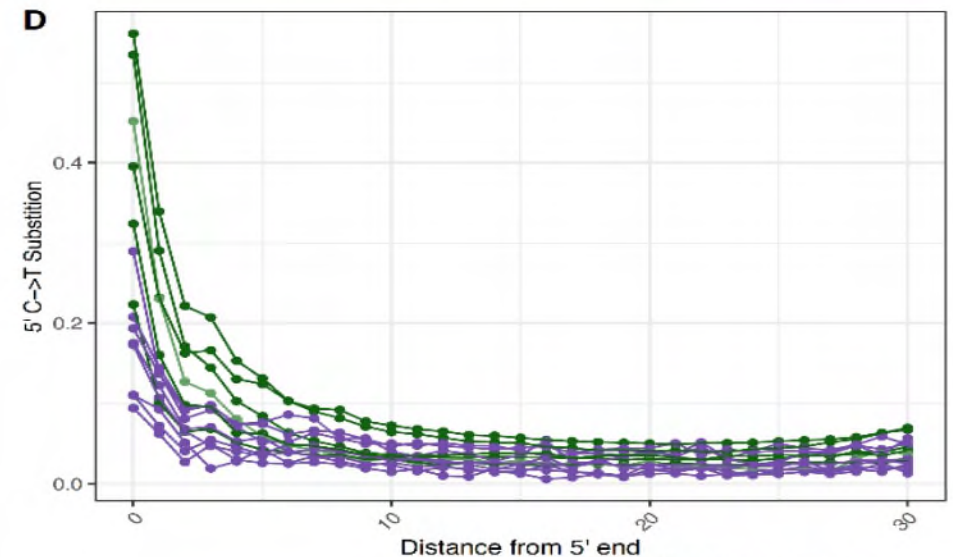




# DNA Quality Assessment



- Comparison of **disinterred remains from WWII/Korean War (purple)** and **ancient DNA up to 40,000 years old (green)**
  - Collaboration with the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany
  - DNA fragment length is similar between DPAA and ancient DNA samples (top)
  - Slightly different damage pattern between the sample sets
    - But DPAA samples had more/similar cytosine deamination (damage) throughout the DNA strand

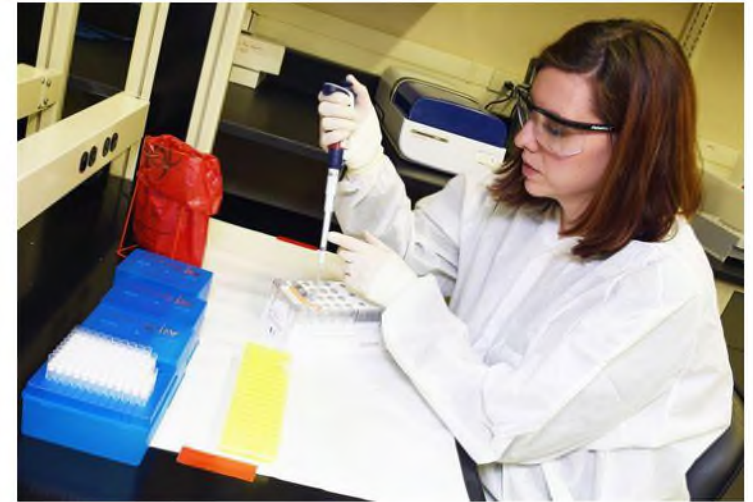




# mtDNA NGS Success



- Processing Capacity Increase to 134 Samples Per Month in 2025
  - 20% increase over 2024
- Since 2016 tested over 7388 samples
- DNA Reports
  - **544 First Time Named Reports/Supports New Identification**
    - ✓ Supported Vietnam, Korea, and WWII cases



<http://www.airforcemedicine.af.mil/Media-Center/Display/Article/1226667/next-generation-dna-sequencing/>



# When Traditional Testing Fails – Where do you go?

Incident	Sample	NGS mtDNA	auSTR	YSTR
CAB	04	Yes	Not tested	Not tested
CAB	05	Yes	Not tested	Not tested
Buna	01	Yes	No Loci	No Loci
Buna	05	Yes	No Loci	No Loci
Korea	05	Yes	No Loci	No Loci
Korea	02	Yes	No Loci	No Loci
Sol Island	03	Yes	No Loci	No Loci
USS WV	03	Yes	No Loci	No Loci
USS WV	04	Yes	No Loci	No Loci



# Single Nucleotide Polymorphism (SNP) Capture Method



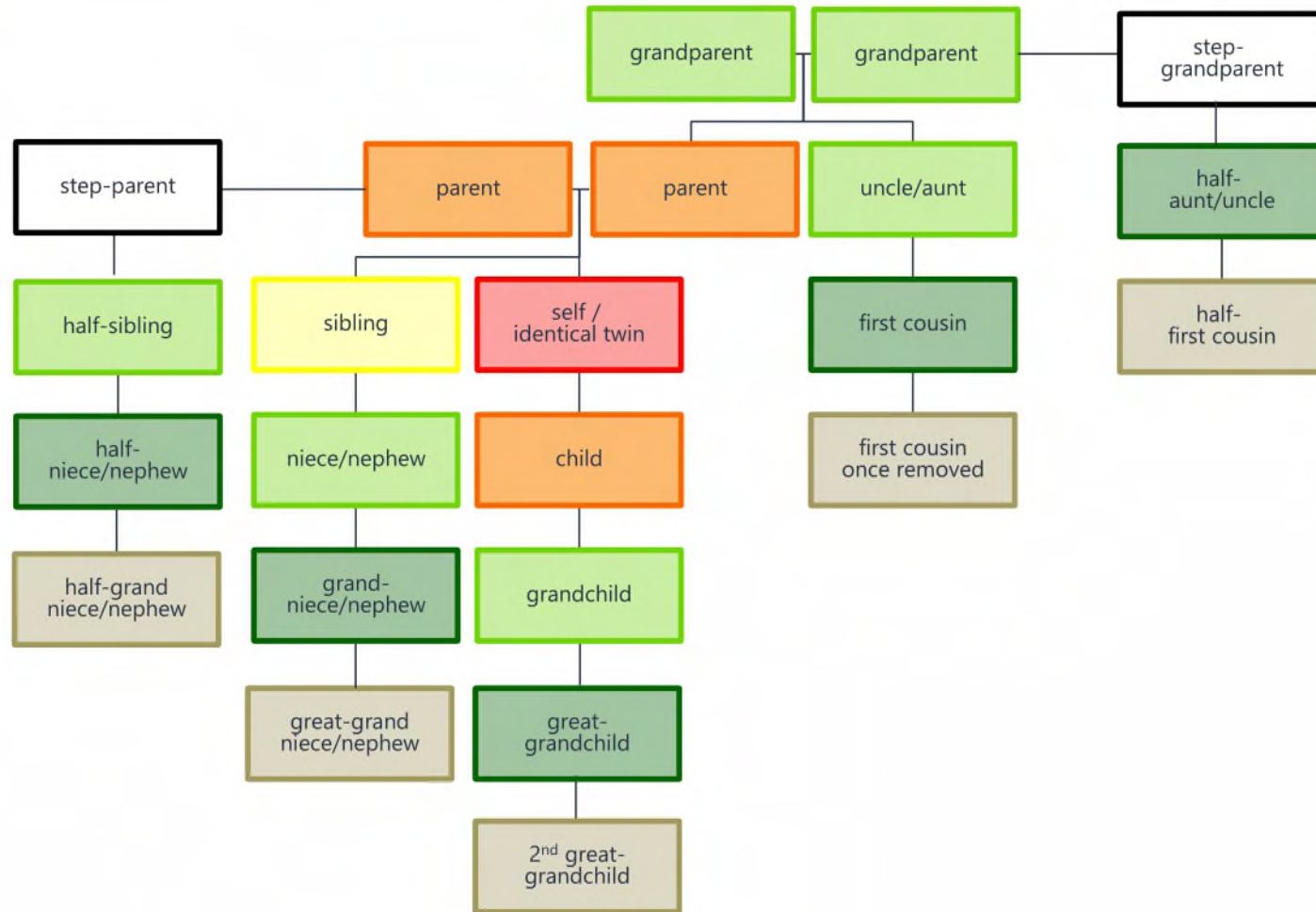
- 2015: Posted Research Request for SNP method and software
- **January 2016: mtDNA capture method brought online**
- 2016: Next Generation Sequencing SNP capture method Research project awarded
- 2016-2024: Development of the method and software
- 2024: Validation of the Next Generation Sequencing SNP capture method
- **January 2025 SNP used to process DPAA casework**



# SNPs expand pool of eligible donors to 4<sup>th</sup> degree relatives

### Relationship

- Self / Identical Twin
- Parent-Offspring
- Full Siblings
- 2<sup>nd</sup> Degree Relatives
- 3<sup>rd</sup> Degree Relatives
- 4<sup>th</sup> Degree Relatives
- Unrelated

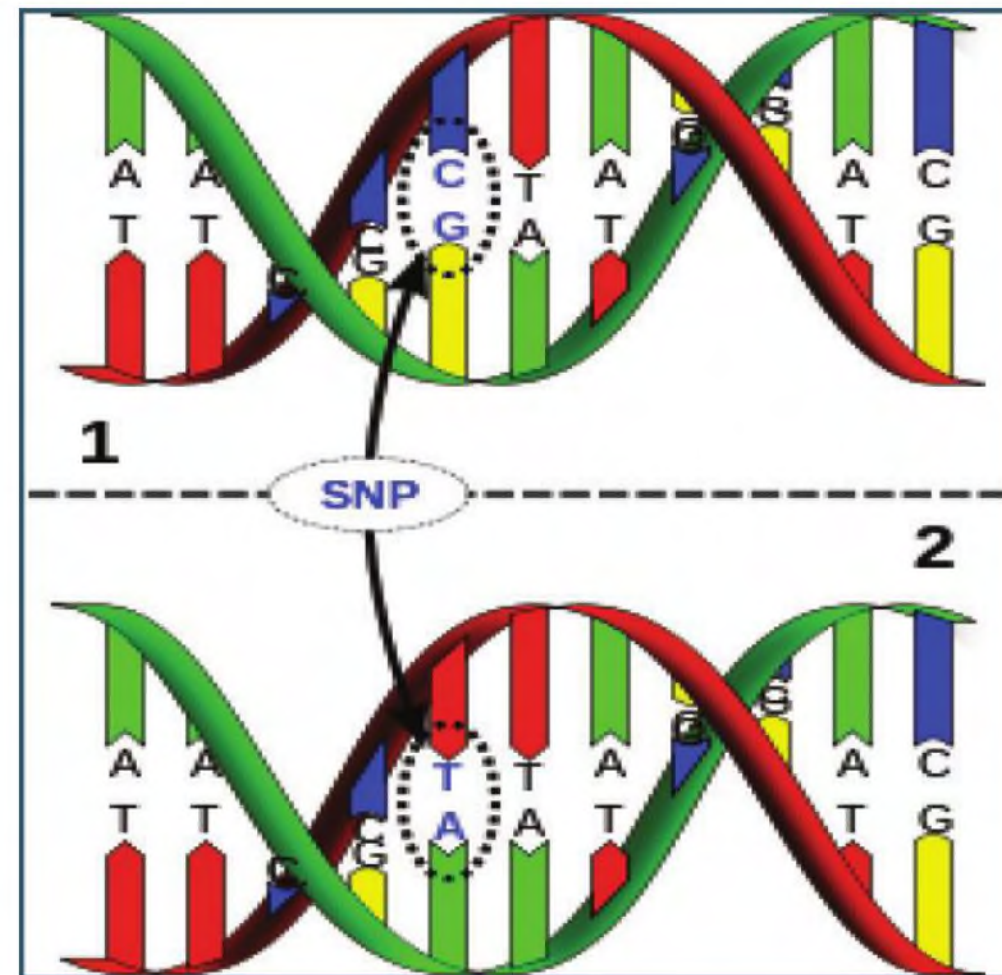




# What is a SNP (Single Nucleotide Polymorphism)?



- Single = one
- Nucleotide = DNA base
- Polymorphism = many forms
  
- SNP = a single DNA base pair that varies in a population



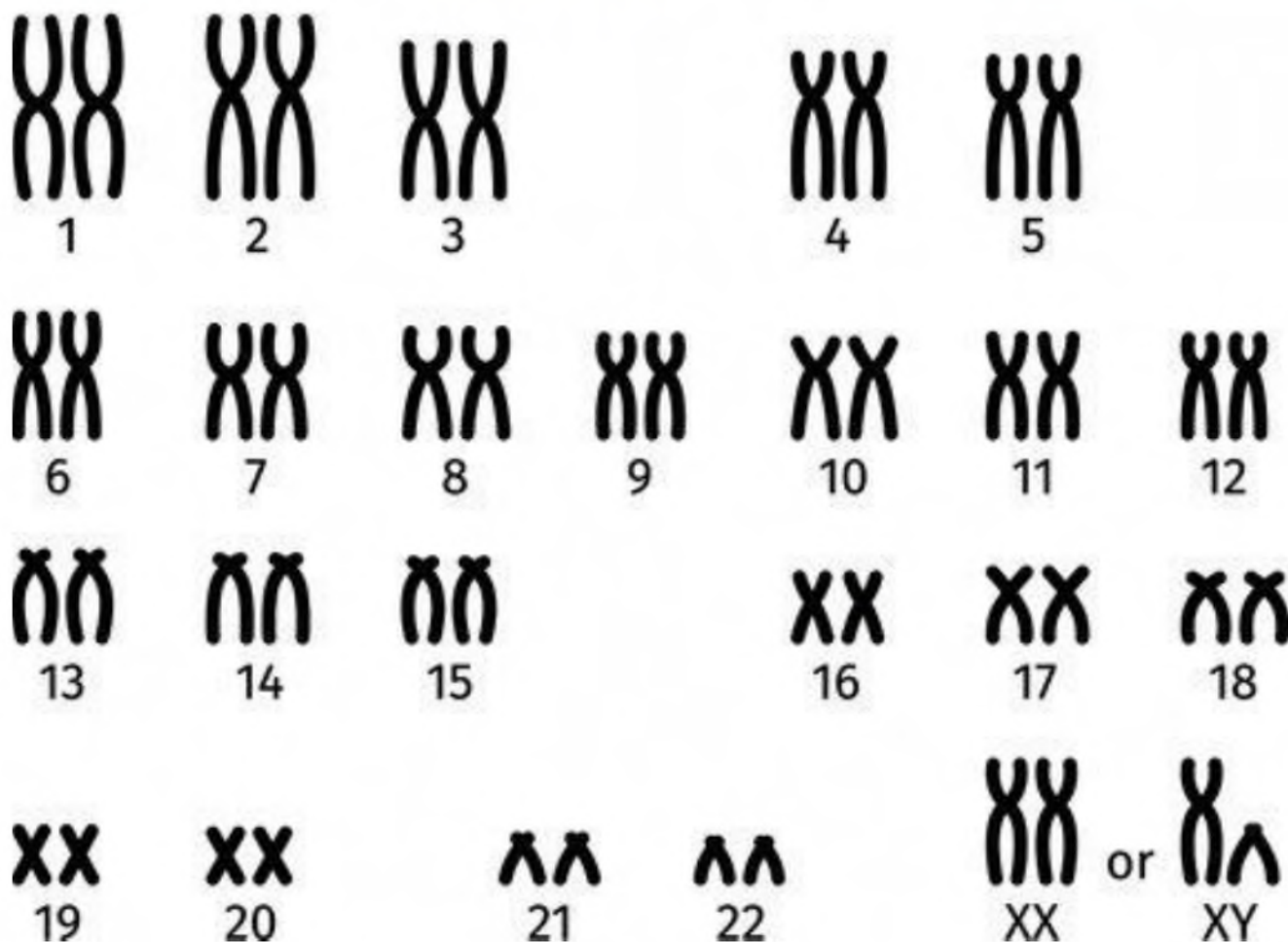
[https://www.researchgate.net/figure/Single-nucleotide-polymorphism-SNP\\_fig1\\_328344689](https://www.researchgate.net/figure/Single-nucleotide-polymorphism-SNP_fig1_328344689)



# 95K SNP Capture Panel



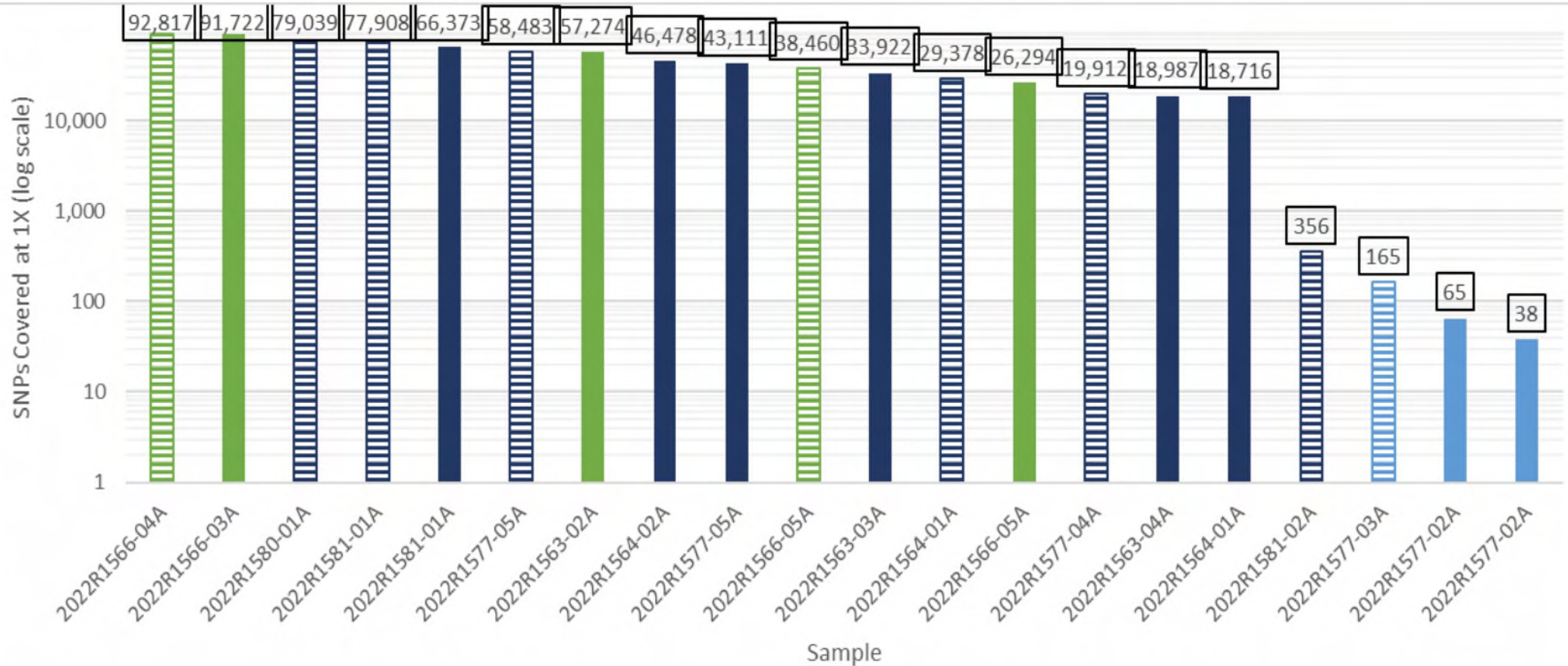
Human karyotype



Chromosome	SNPs	% of Total
chr1	7886	8.3%
chr2	7405	7.8%
chr3	6061	6.4%
chr4	4770	5.0%
chr5	5651	6.0%
chr6	6697	7.1%
chr7	5179	5.5%
chr8	4766	5.0%
chr9	4428	4.7%
chr10	5657	6.0%
chr11	4672	4.9%
chr12	4249	4.5%
chr13	3411	3.6%
chr14	3411	3.6%
chr15	2916	3.1%
chr16	3221	3.4%
chr17	3051	3.2%
chr18	2933	3.1%
chr19	1835	1.9%
chr20	2493	2.6%
chr21	1403	1.5%
chr22	1464	1.5%
chrX	448	0.5%
chrY	745	0.8%
<b>Total</b>	<b>94752</b>	<b>100.0%</b>



# SNP Validation Results



Sanger Sequence

NGS Sequence

Inconclusive

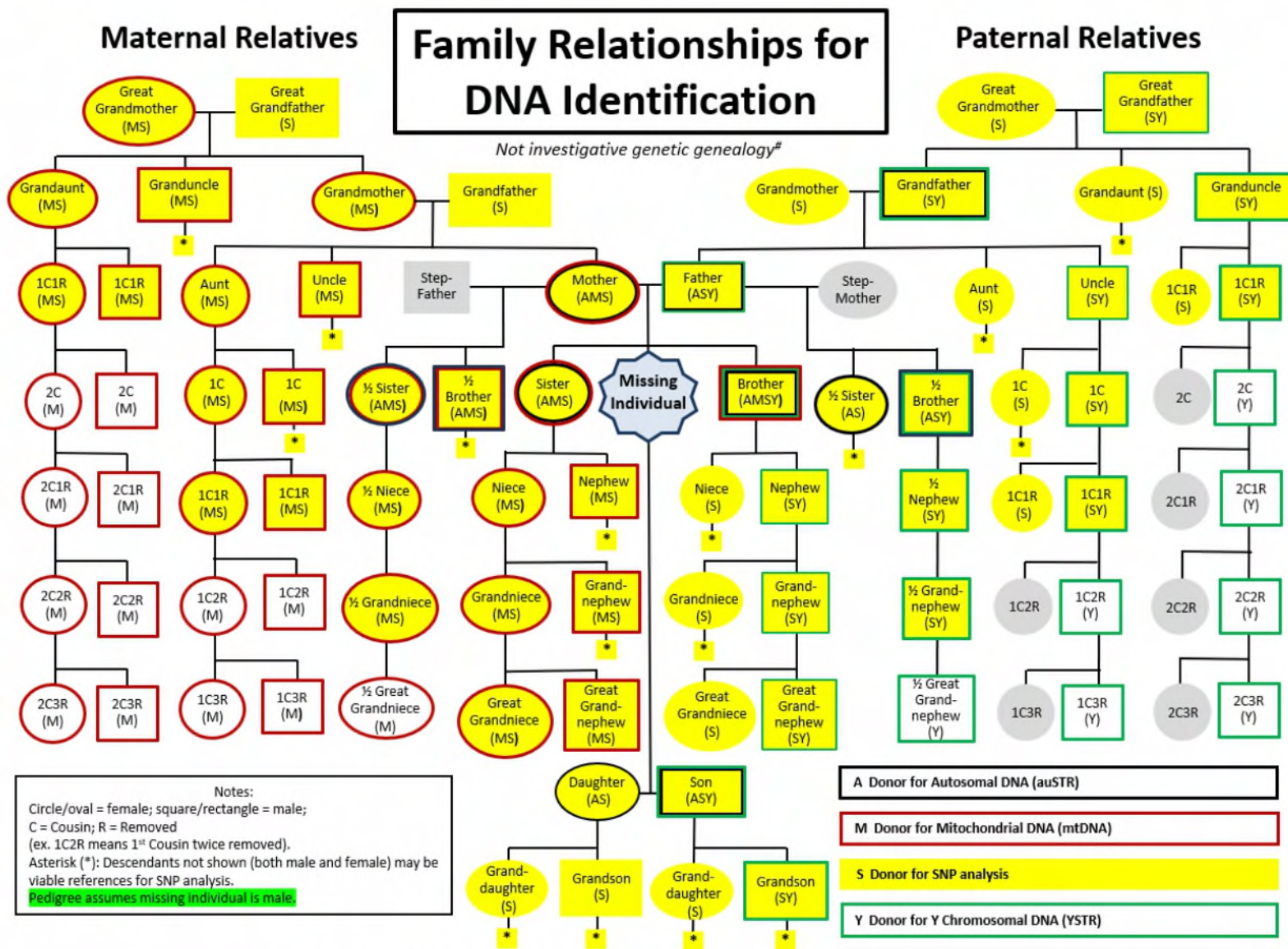


# SNP Capture Results – World’s Population 8E<sup>9</sup>

Incident	Sample	NGS mtDNA	auSTR	YSTR	95K SNP	SNP-REF
CAB	04	Yes	Not tested	Not tested		
CAB	05	Yes	Not tested	Not tested		
Buna	01	Yes	No Loci	No Loci		
Buna	05	Yes	No Loci	No Loci		
Korea	05	Yes	No Loci	No Loci		
Korea	02	Yes	No Loci	No Loci		
Sol Island	03	Yes	No Loci	No Loci		
USS WV	03	Yes	No Loci	No Loci		
USS WV	04	Yes	No Loci	No Loci		



# SNP Viable Family References



#Forensic investigative genetic genealogy is a technique that searches for distant relatives but is not an accredited method for identification. The relationships in this diagram can support DNA identification using accredited forensic DNA testing methods.



# Casework Processing



- Casework processing started February 2025
- First ID with SNPs in March 2025
- Processing numbers to date:
  - SM samples processed: **91**
    - ✓ 87 bones, 4 teeth
    - ✓ Success rate: ~**95%**
  - FRS processed: **280**
    - ✓ Success rate: ~**97%**
  - # ID reports
    - ✓ Complete: **40**
    - ✓ In progress: **18**

DEFENSE POW/MIA ACCOUNTING AGENCY  
FULFILLING OUR NATION'S PROMISE

HOME FAMILIES OUR MISSING NEWS & STORIES MULTIMEDIA RESOURCES ABOUT

RECENT NEWS AND STORIES

News & Stories

**Ens EUGENE E MANDEBERG**

NEWS | March 21, 2025

**New DNA Technology Helps Identify Missing WWII Aviator**

By Staff Sgt. David Owsianka  
Defense POW/MIA Accounting Agency Public Affairs



# AFMES-AFDIL Forensic DNA Testing Methods



	Mitochondrial DNA – Sanger Sequencing**	Nuclear DNA Low-Copy Number – YSTR*	Nuclear DNA Autosomal STR*	Mitochondrial DNA – Next Generation Sequencing***	Nuclear DNA Single Nucleotide Polymorphism (SNP)***
Fit for use?	Yes. Excellent for degraded DNA – non chemically treated	Maybe. Not great for degraded DNA.	Maybe. Not great for degraded DNA/chemically treated.	Yes. Excellent for degraded DNA/chemically treated	Yes. Excellent for degraded DNA.
Discrimination	Excellent sorting tool, <b>Maternal</b> lineage marker <b>good STR success</b>	Excellent sorting tool, <b>Paternal</b> lineage marker; good for combined statistic	Excellent! Positive form of identification with <b>appropriate Reference</b>	<b>Maternal</b> Lineage marker, <b>limited STR success</b>	<b>Positive form</b> of identification. Expands FRS.
Effort	LOTS of hands on. Completely manual.	Limited hands on but can be automated.	Hands on but can be automated	Moderate hands on. Much is automated; allows batching samples	Moderate hands on but allows for batching.

\* Traditional test used in crime laboratories

\*\* Limited in Crime Laboratories

\*\*\* **Only accredited laboratory with these methods**



# Forensic Genetic Genealogy or Investigative Genetic Genealogy



- **Genetic Genealogy:**
  - Who am I related to; What potential medical issues may I have; What is my heritage
- **Forensic Genetic Genealogy:**
  - Criminal/Missing person Cold Case
  - Assisting with solving State and Local cold case.
    - Investigative lead; must confirm with STR or mtDNA sequencing
  - FamilyTreeDNA and GEDmatch allow law enforcement searches
    - Privacy issues; time consuming
  - Requires a lot of DNA; non-human DNA cross reacts
- **DPAA Casework Scenario:**
  - All FRS have been excluded or there are no reference available
    - Adoptions, relationship issues, genealogy fails to identify a viable reference, current reference greater than 4<sup>th</sup> degree relative



# Bone – SNP Recovery and Coverage



Sample	FORCE - SNP (1X)	AFDIL 95K -SNP (1X)	Kintelligence (10X)	WGE (Avg Cov)	WGS (Avg Cov)
B1.1	1574	12,123	444	0.35	0.00
B1.2	364	5638	-	0.07	0.00
B2.1	17	327	-	0.00	0.00
B2.2	3070	36,381	480	0.25	0.00
B3.1	4653	82,460	3677	3.54	0.01
B3.2	5364	94,299	-	5.54	0.00

\*\*\*NO data generated is GEDmatch uploadable (insufficient number of SNP genotypes):  
GEDmatch upload required >150,000 SNPs or 6,000 SNPs from Kintelligence

All European ancestries - SNP



# Samples



- **Case 1**
  - 2 bones (B1 and B2)
  - 1 potential SM with 2 FRSs
    - ✓ Maternal 2<sup>nd</sup> cousin (5<sup>th</sup> degree) [R1]
      - Not SNP eligible; mitochondrial eligible
    - ✓ “Paternal” 1<sup>st</sup> cousin (female) (3<sup>rd</sup> degree) [R2]
      - SNP eligible; not mito or Y eligible
  - ❖ **Potential SM excluded based on >4 differences across the mitogenome**
- **Case 1**
  - Confirmed Bone 1 and Bone 2 are from the same individual
    - Self LR:  $7.89 \times 10^{255}$
    - Self PP: ~100%
  - Excluded FRS 2 as a close relative of Bone 1/Bone 2
    - Unrelated PP: ~100%
    - 3<sup>rd</sup> Deg LR (PP):  $3.40 \times 10^{-62}$  (~0%)
  - Supported the mitochondrial genome results,
  - Commercial FIGG kits need to be optimized



# What Does This Mean To You



- DNA testing of human remains is complicated and case dependent
- AFMES-AFDIL is using cutting edge technology
  - Next Generation Sequencing Methods
- AFDIL does not use a limited set of DNA testing Methods
  - Keep all validated methods new and old online
  - Allows AFMES-AFDIL to adapt to changing sample conditions
- Continually monitor success rates, reassess, and optimize
- Revisit past failures with new techniques – First time through may not always be the best.
- Continue to be a world leader in the development of Forensic human remains DNA testing methods.



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Pictures taken by SSgt Devon Schultz AFMES



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# QUESTIONS & ANSWERS



DEFENSE POW/MIA ACCOUNTING AGENCY

# Closing Remarks

Mr. Kelly McKeague  
Director, DPAA

*Vietnam War Annual Government Briefing 26-27 June 2025*



**AGB Survey**