Sandia National Laboratories

SAND2018-10656C

Real-time human health and performance monitoring

RIM-TO-RIM WEARABLES AT THE CANYON FOR HEALTH
R2R WATCH STUDY

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U.S. DEPARTMENT OF ENERGY
NNSA National Nuclear Security Administration

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DEFENSE THREAT REDUCTION AGENCY
UNITED STATES OF AMERICA

Sandia's Mission Work Reflects National Security Challenges

1950s
NW production engineering & manufacturing engineering

1960s
Development engineering
Vietnam conflict

1970s
Multiprogram laboratory
Energy crisis

1980s
Missile defense work
Cold War

1990s
Post−Cold War transition
Stockpile stewardship

2000s
Expanded national security role post 9/11

2010s
LEPs
New START
Evolving national security challenges

Need for Cross-Domain Situational Awareness

Problem: Warfighter's environment changes quickly, and ability to respond rapidly and effectively can mean life or death for themselves and/or their team.

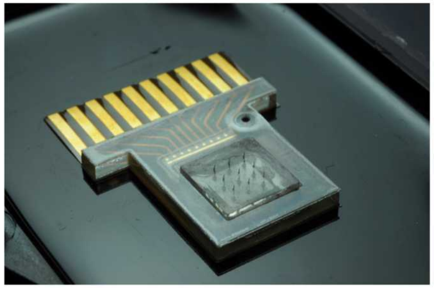
Solution : Passive, wearable and continuous monitoring strategies to assess and understand warfighter status from multiparameter data (context, vitals, and chemistry)

Requires:

- Multiparameter data streams
- Rapid data relay, analysis and integration
- Rapid actionable response

Two DTRA projects:

Transdermal Microneedle Sensors to Measure Biomarker Signatures and Report on Human Performance



Ronen Polsky
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Wearables At The Canyon for Health (WATCH): A human-subjects study on wearable devices

Principal Investigator: Glory Aviña, PhD MBA

Microneedles Enable Health Monitoring

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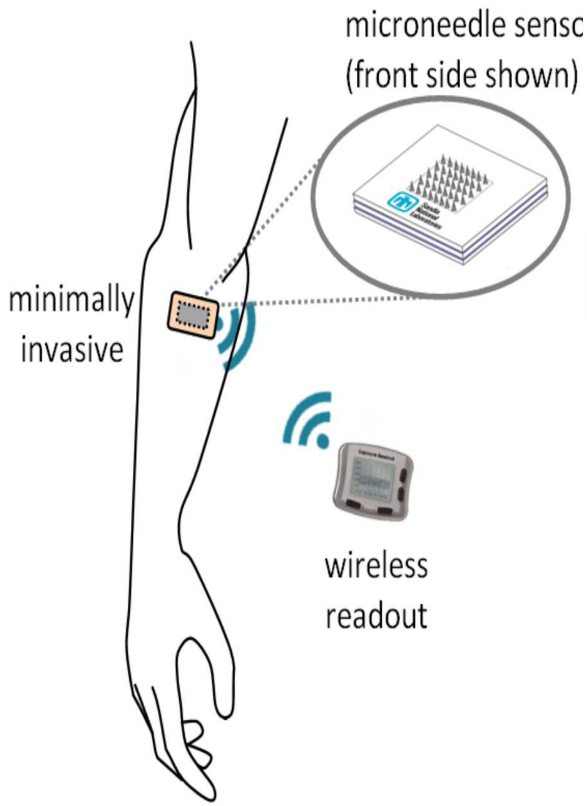
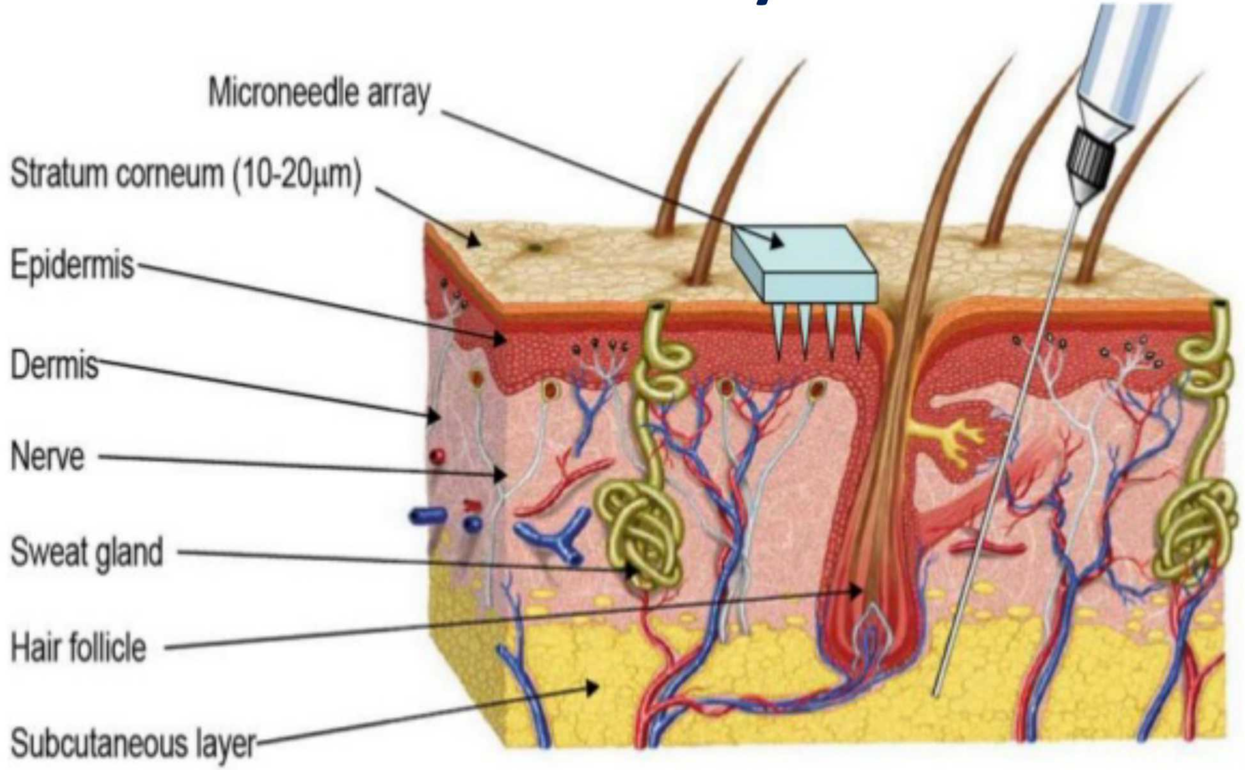
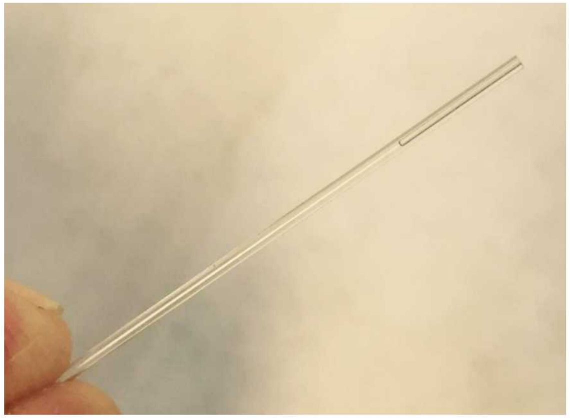


- Microneedle platform integrated Raman detection to monitor physiology.
- Multiplexed sensing platforms allows for site specific and systemic monitoring of biological markers indicative or radiation exposure
- Device to be worn directly on skin for transdermal sensing.
- Electronic readout can be used to send results back to a central command station for remote monitoring.
- The real time analysis of these biomarkers will allow immediate determinations of necessary counter measures and be used for triage situations.

Why Microneedles



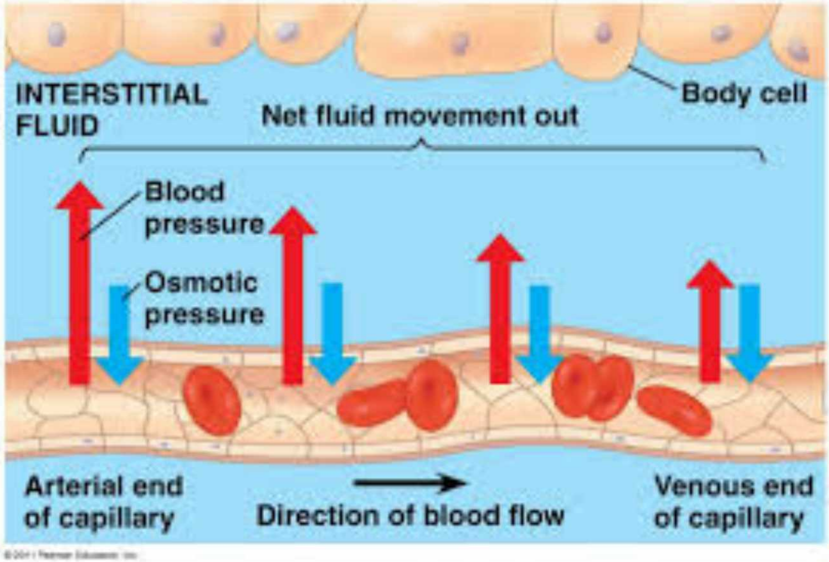
Extracted IF

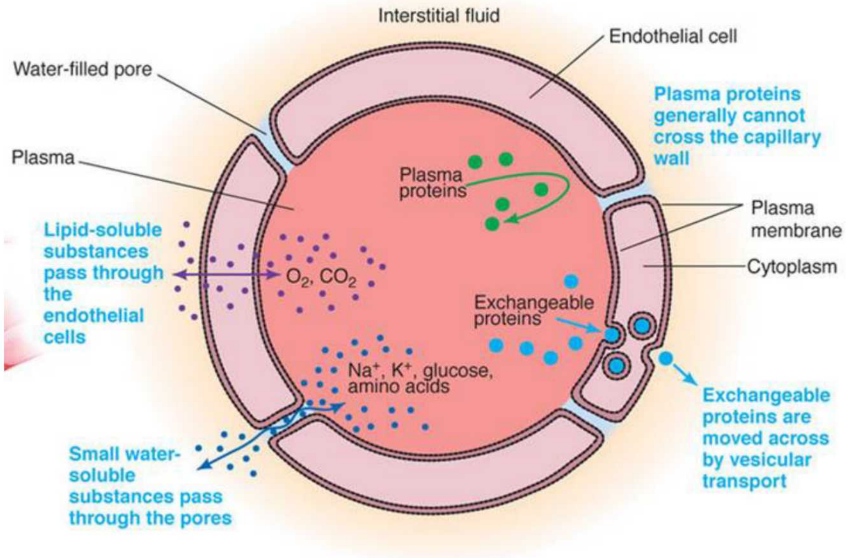


- Microneedles do not reach nerve endings so are painless
- Interstitial fluid (the extracellular fluid between cells) and not blood is extracted which has a rich concentration of available biomarkers for physiological interrogation

Microneedles can provide minimally invasive, pain free access to biomarkers for wearable health monitoring applications.

Interstitial fluid is a form of plasma filtrate



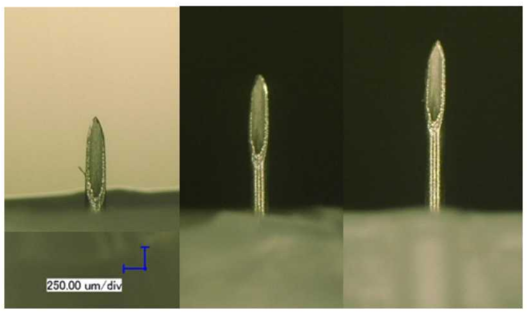


Transport across capillary wall

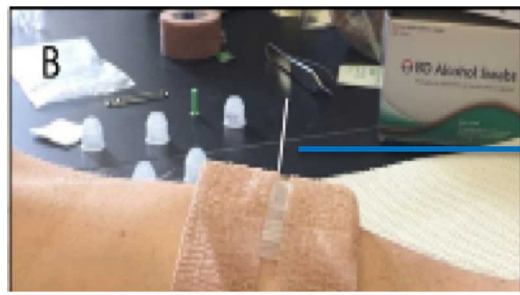
Extraction Protocol

- Three different needle lengths were examined for extraction efficiency (volume ISF extracted) and pain sensation.
- After maximum 2 hours insertion no visible immune response was observed
- ***<u>Needles are modified from insulin injectors FDA approved and biocompatible</u>***

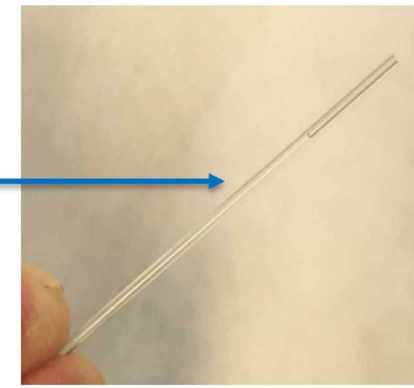
1000um 1500um 2000um



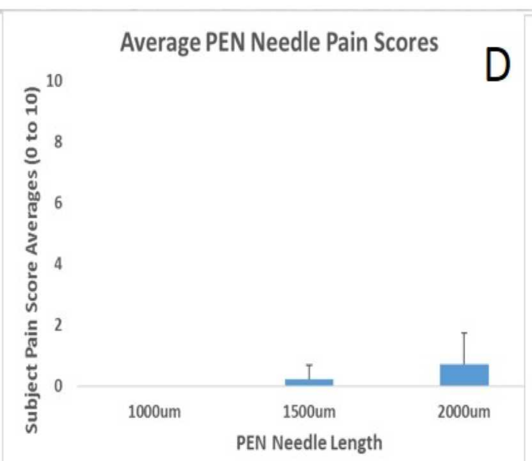
Varying PEN needle lengths cut at different lengths



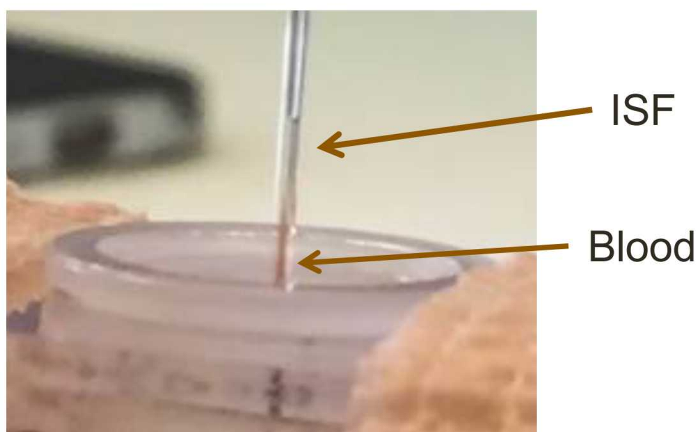
Clinical Format



Extracted Fluid



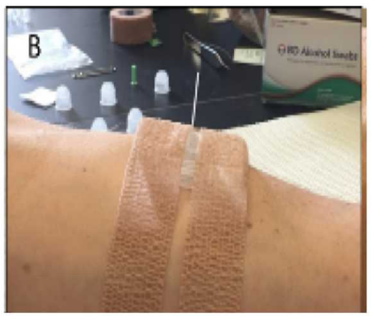
Pain Scores vs Length



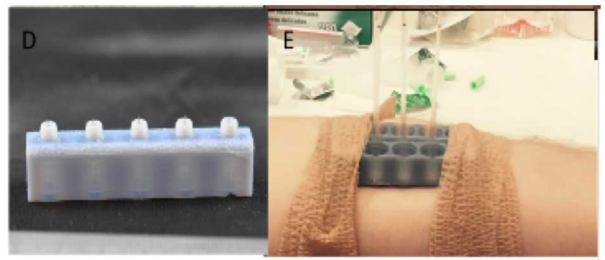
2000 μm Needle (pain sensation 4/10)

- Biocompatible needles
- Novel method for ISF extraction
- Pain free
- Optimum length: 1500 μm

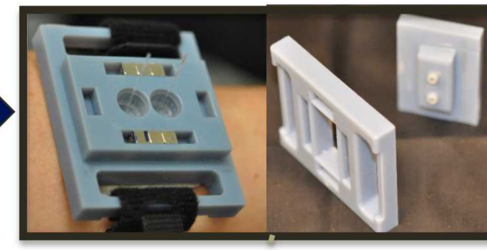
Holder Development



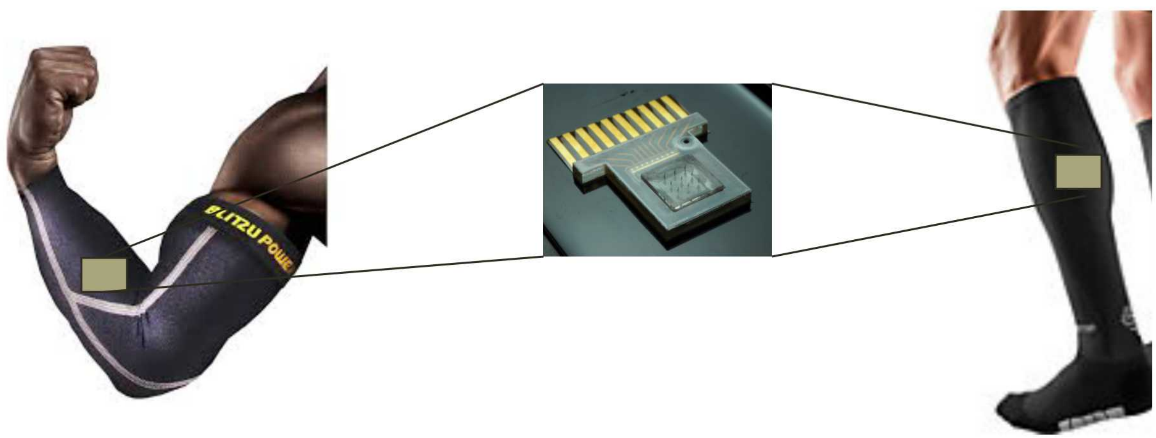
Generation 1:
single needle
1-2 µl ISF



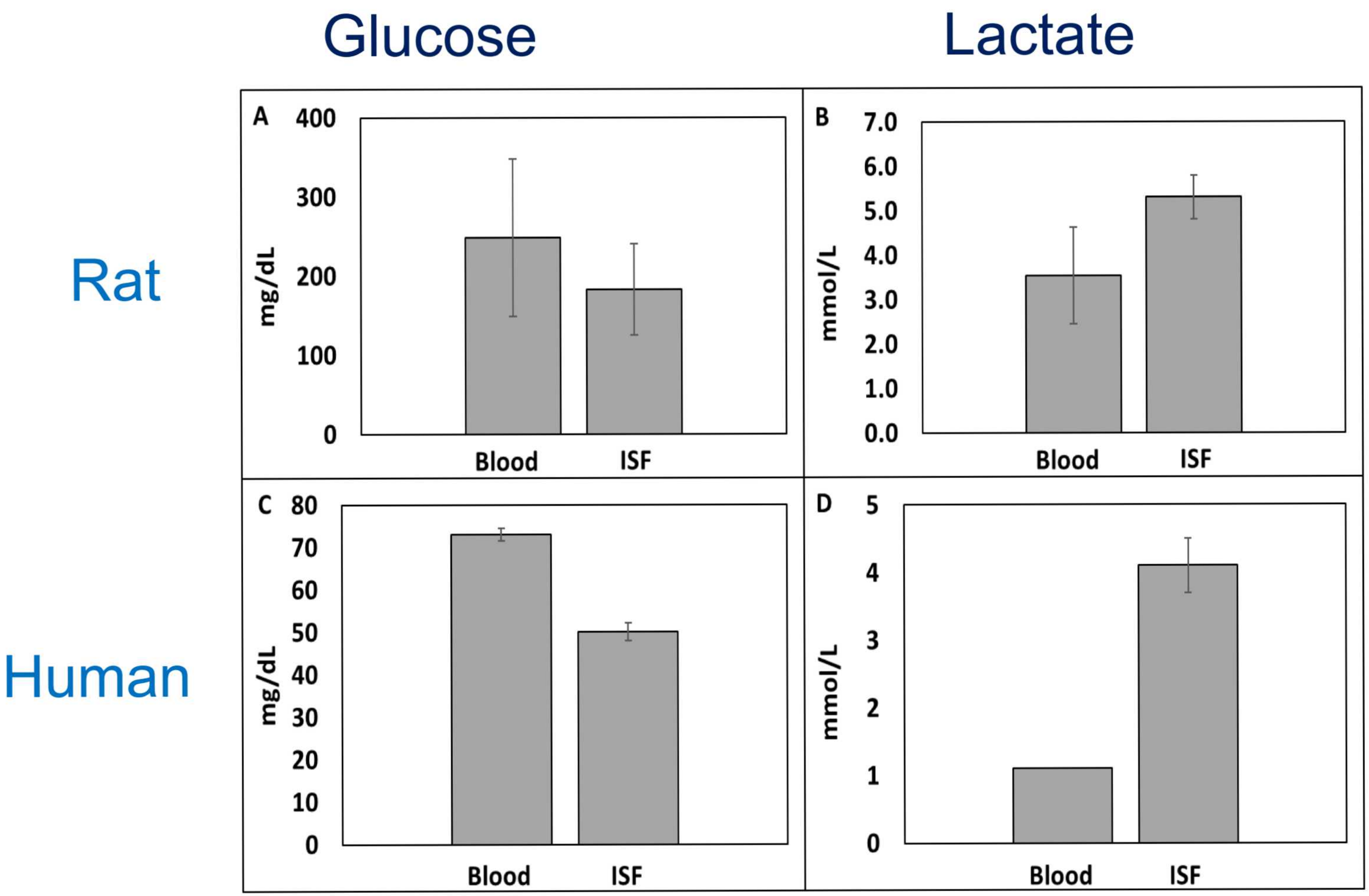
Generation 1: Arrays
5-20 µl ISF Humans
40-50 µl ISF Rats



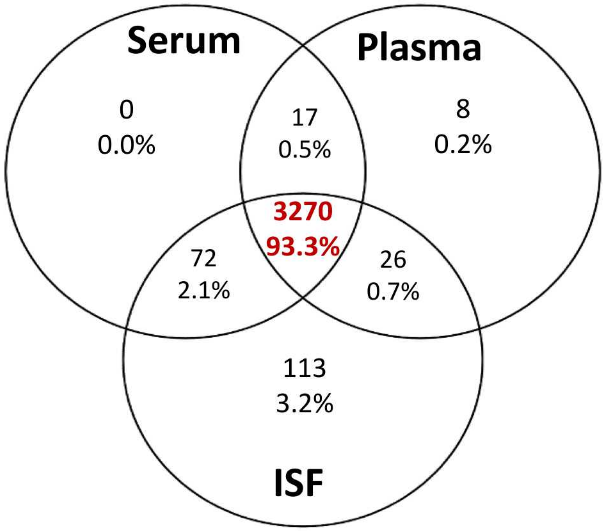
Generation 3: Wearable
Removable Cartridge
Hands free

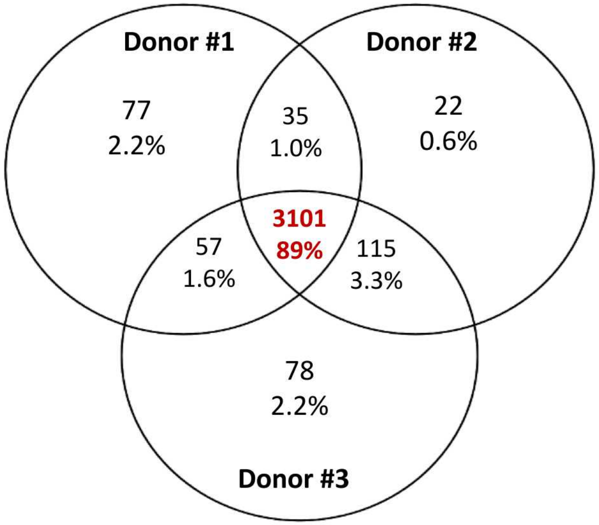


Metabolite Data



Proteomics Analysis: ISF is Proxy for Serum





- Of 3506 proteins identified in human subject, 3270 proteins were common between plasma, serum and ISF

- 93% match in protein content

- Indicates ISF can be used as a blood proxy

- 89% of these proteins were consistently detected in three examined human individuals.

J. Proteome Res. 2018, 17, 479–485

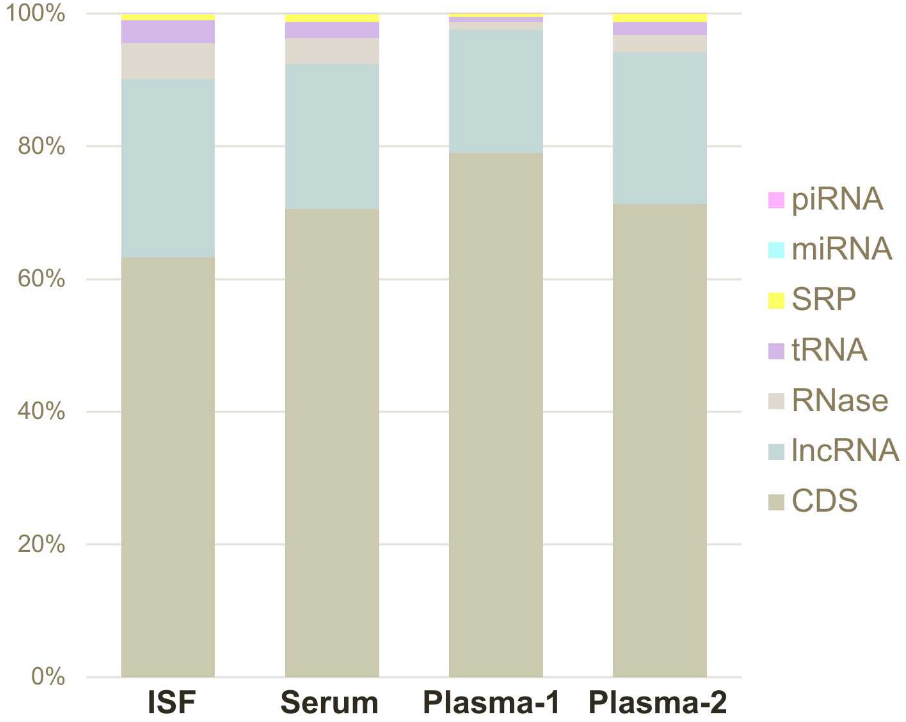
In collaboration with Trevor Glaros and Nicole Rosenzweig, ECBC

Transcriptomics

A.

	ISF		Serum		Plasma-1		Plasma-2	
Mapped Reads (M)	1.9	(1.7 - 4.8)	2.2	(1.3 - 5.6)	4.9	(2.3 - 6.8)	3.7	(2.5 - 4.8)
CDS (%)	63.2	(22.3 - 84.2)	70.6	(41.1 - 87.9)	78.9	(71.5 - 88.8)	71.4	(62.1 - 76.9)
lncRNA (%)	26.9	(7.4 - 74.4)	21.8	(7.1 - 52.3)	18.6	(7.2 - 26.6)	22.9	(18.8 - 28.0)
RNase (%)	5.4	(1.4 - 11.3)	3.9	(0.4 - 5.3)	1.2	(0.4 - 2.6)	2.6	(0.5 - 5.1)
tRNA (%)	3.5	(1.3 - 5.7)	2.4	(1.4 - 4.2)	0.7	(0.4 - 1.4)	1.9	(0.7 - 3.0)
SRP (%)	0.9	(0.2 - 2.6)	1.2	(0.2 - 3.2)	0.5	(0.3 - 0.7)	1.2	(0.6 - 1.7)
miRNA (%)	0.05	(0.01 - 0.09)	0.08	(0.04 - 0.13)	0.03	(0.01 - 0.05)	0.05	(0.01 - 0.10)
piRNA (%)	0.04	(0.00 - 0.07)	0.03	(0.01 - 0.06)	0.01	(0.00 - 0.01)	0.01	(0.00 - 0.02)

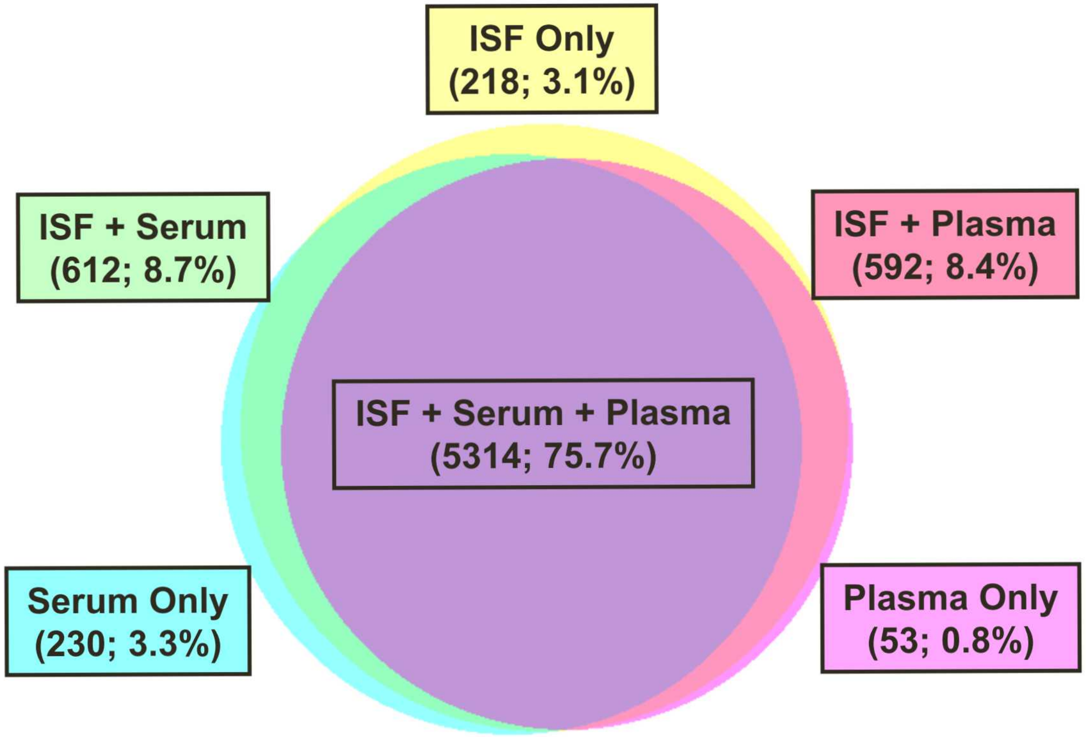
B.



RNA species detected in ISF, serum, and plasma samples

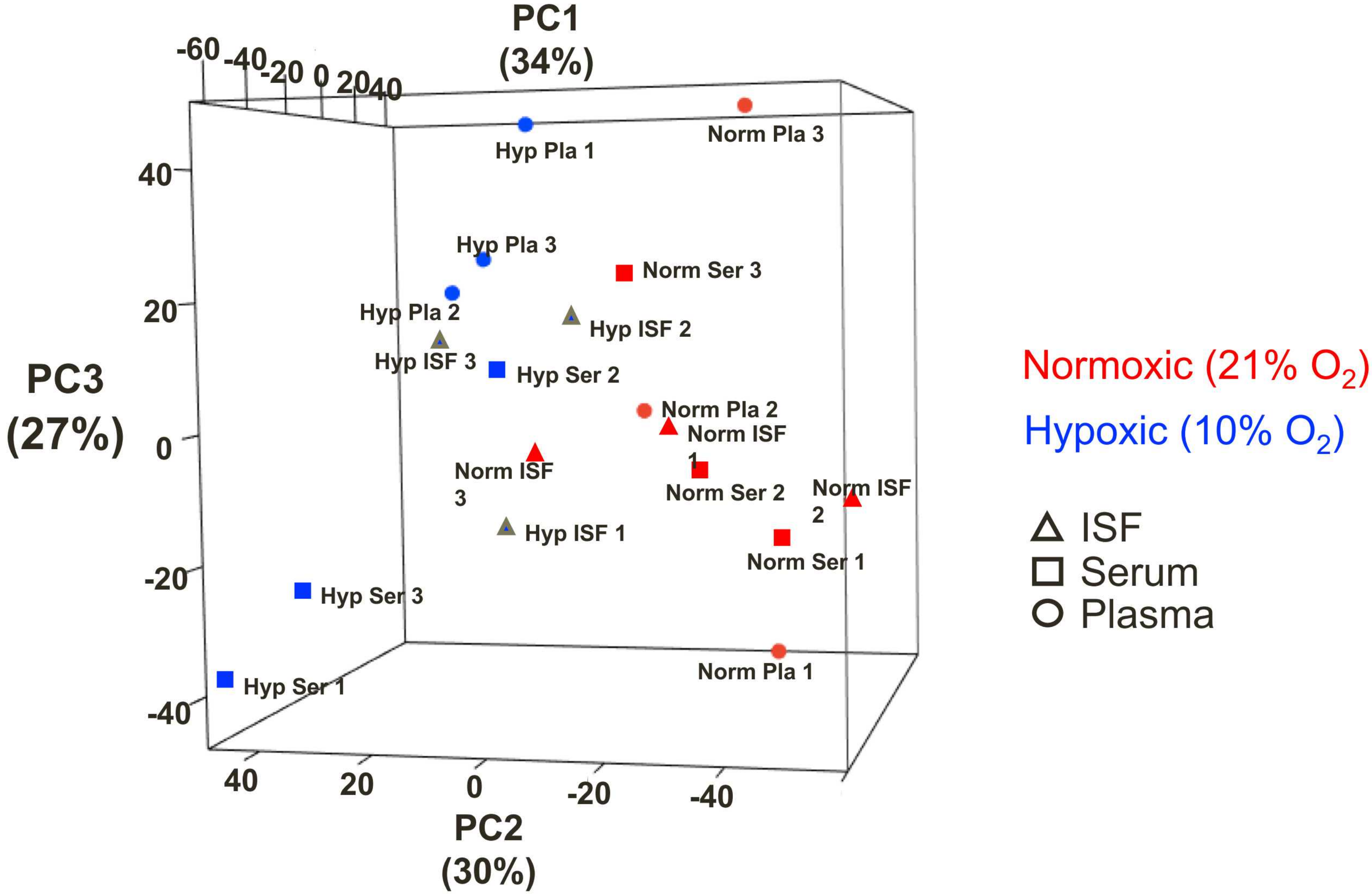
Transcriptomics Analysis: ISF is Proxy for Serum

CDS that were hit in each sample type in 4 rats



75% of coding sequence RNAs were commonly identified

Dynamic Response of ISF Contents to Physiological Stress



Physiological changes are seen in all 3 fluids after induced hypoxia

Clinical Significance

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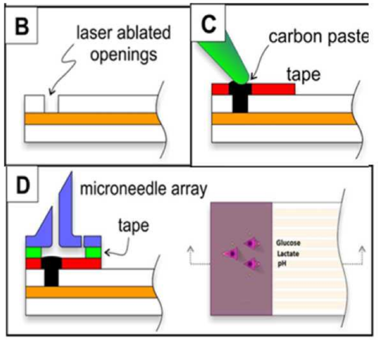
Similar to previous studies of plasma content, annotated functions of genes encoding transcripts are differentially expressed in ISF and can be monitored to diagnose hypoxia

Function	Genes
Matrix Metalloproteinases (MMPs)	Mmp8, Mt1
Chemokines	Cxcl13, Ccr1
Leukocyte Migration	Stk10
Innate Immunity	Hmgb1-ps3, Lacc1, Bnip1
Transcription Regulator	Creg1, Brwd3 Mecp2, Sertad2, Tada2b, Tle1
Mitochondrial Processes	Pcca, Tomm40, Mtx1
Heat Shock Protein Regulation	Dnajb9
Cytoskeleton and Cell Adhesion	Mpzl2, Actn3
DNA Repair	Tdg

Transcripts that are enriched (red) or depleted (blue) in ISF in rats experiencing hypoxia (10% oxygen), as compared to rats experiencing normoxia (21% oxygen).

Nature Communications Biology – in revisions

Sensor Development



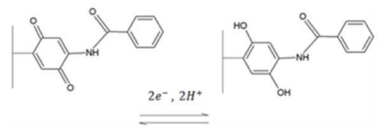
Needle 1: $Lactate + H_2O + O_2 \xrightarrow{LOX} Pyruvate + H_2O_2$

$H_2O_2 \xrightarrow{RhonC\,@-0.15V} 2H + O_2 + 2e^-$

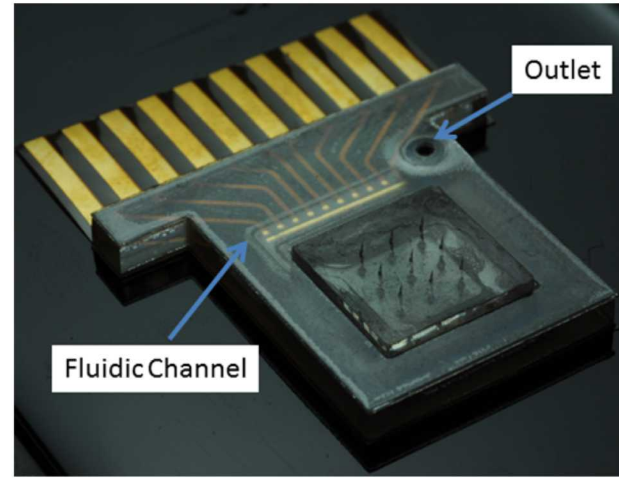
Needle 2: $Glucose + H_2O + O_2 \xrightarrow{GOX} Gluconicacid + H_2O_2$

$H_2O_2 \xrightarrow{RhonC\,@-0.05V} 2H + O_2 + 2e^-$

Needle 3:

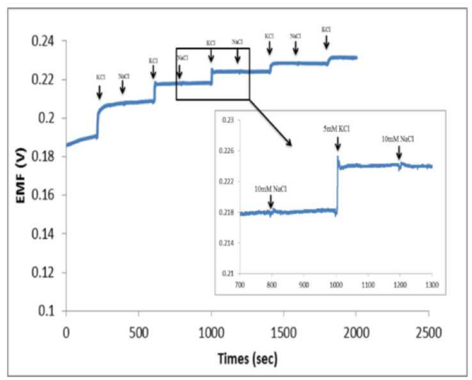


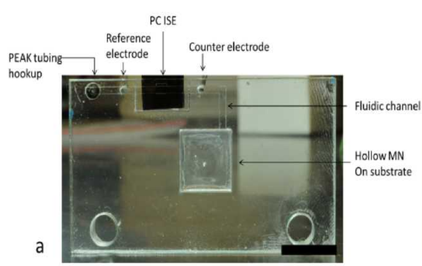
Multiplexed Microneedle Array

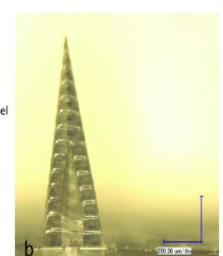


Protein Chip

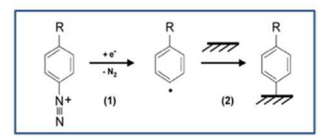
Microneedle K⁺ Sensor

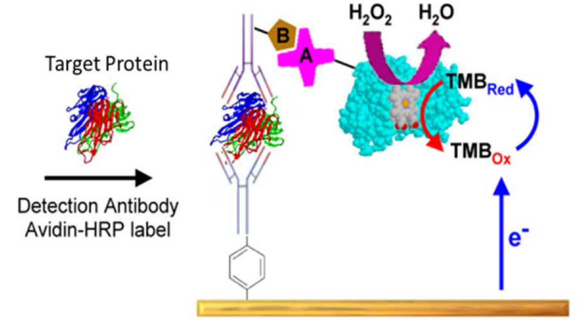






Diazonium Modification





SNL Project 164370
SNL Proposal 017120403
DTRAJ9-CXT Innovative Research Program
PM: Bruce Hinds

Microneedle Electrode

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Coaxial Referenced PEN Needle Biosensor

- **<u>Goal:</u>** Adapt the PEN needle assembly for an enzymatic lactate biosensor with a built-in reference electrode
- **<u>Tasks:</u>**
  - Insulate PEN needle
  - Apply carbon paste enzymatic biosensor at needle tip
  - Electrical connection to biosensor

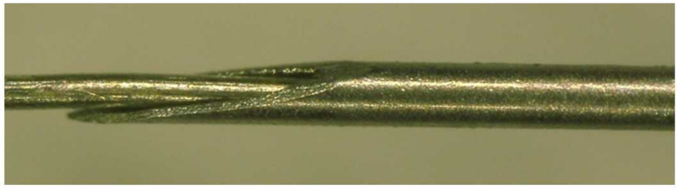
Top-Down View of Sensor

- Biosensor
- Dielectric
- PEN Needle
- Reference Electrode

PEN Needle w/Sensor

- **<u>Electrical connection to biosensor:</u>**

-Pastes are too viscous to force down needle bore, and diluted graphite powder to mineral oil ratios electrodes with poor electrochemical properties.
-Cu allow wires both fill needle bore and provide suitable electrical connection. Electrical resistance ~0.1Ohm (1” length).



Wire inserted within PEN needle. Positioned well beyond needle tip to show size comparison.

Modified PEN Needle

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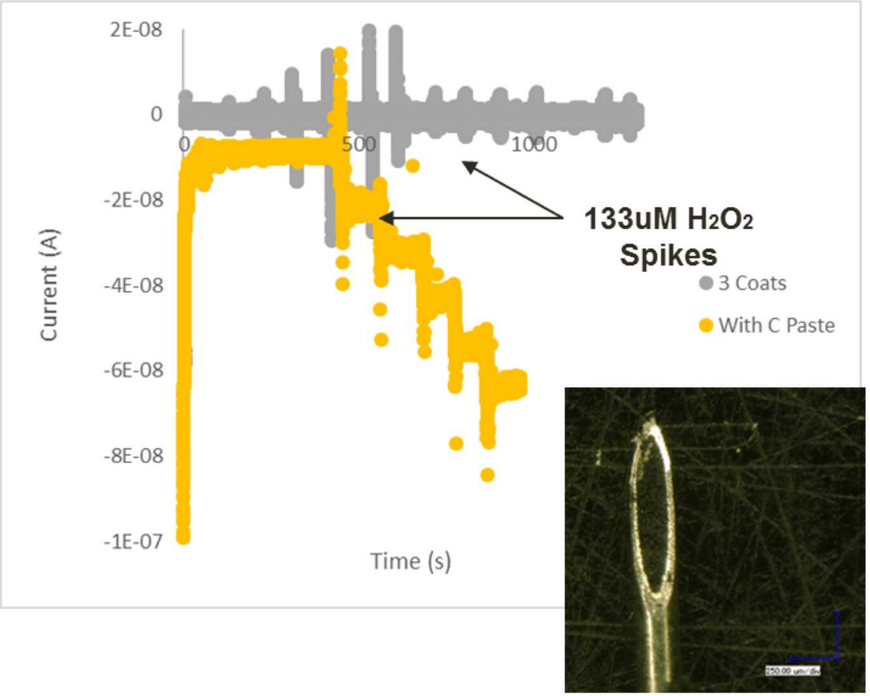
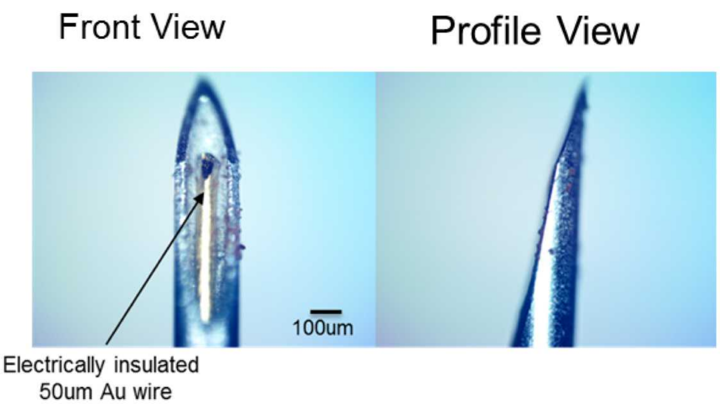
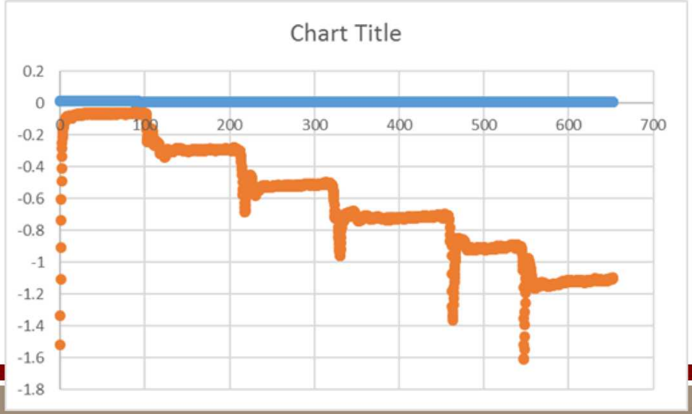
Converting commercial needle into electrode transducer simplifies sensor construction. Requires no advanced fabrication and uses readily available materials.





- Created method for protecting electrode within needle
- Can be used for reference and/or working electrode
- Wire electrically isolated from needle
- Each needle is a sensor and large arrays of individually addressable sensor possible

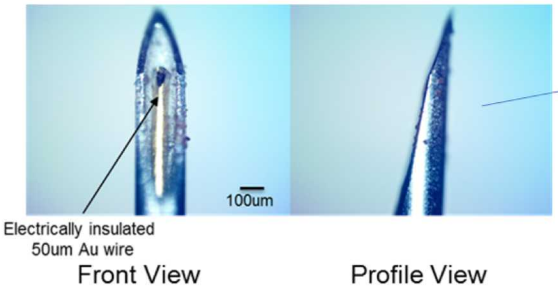
Carbon Paste Packed Needle



- Tested the response to peroxide on a insulated needle (grey, 3 coats) and an insulated needle with carbon paste packed at the tip (yellow)
- Insulated needle isn't reactive to the test solution.
- Adding LOx to the paste will create the enzymatic lactate biosensor

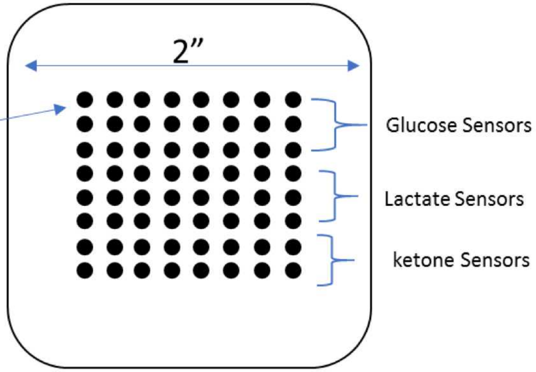
Multiplexed Microneedle Sensor

Individual Microneedle Sensor



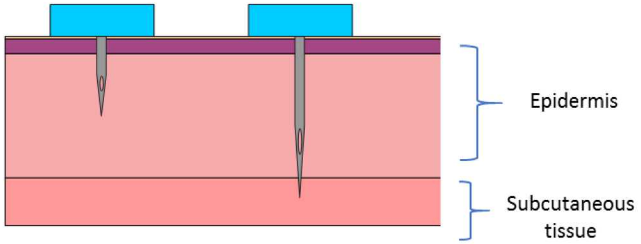
-Each coaxial sensor can self-reference
-Potential for multiple sensors per needle

Array of Sensor on Wearable Patch



-Each row of sensors can be customizable to the analyte of interest
-Suitable sample sizes can help determine System flux
-Compatible with suite of sensors and ISF fluid extraction microneedles

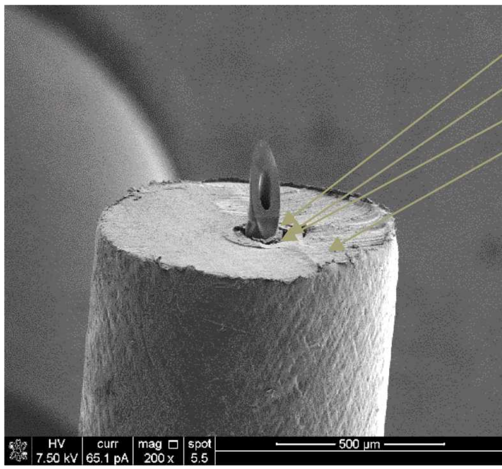
Sensor Placement Depth Control



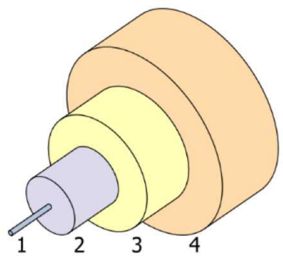
-Microneedle sensor depth control allows monitoring different skin layers
-May help distinguish physiological responses that are driven by external factor or via internal ones

Microneedle Optical Sensor

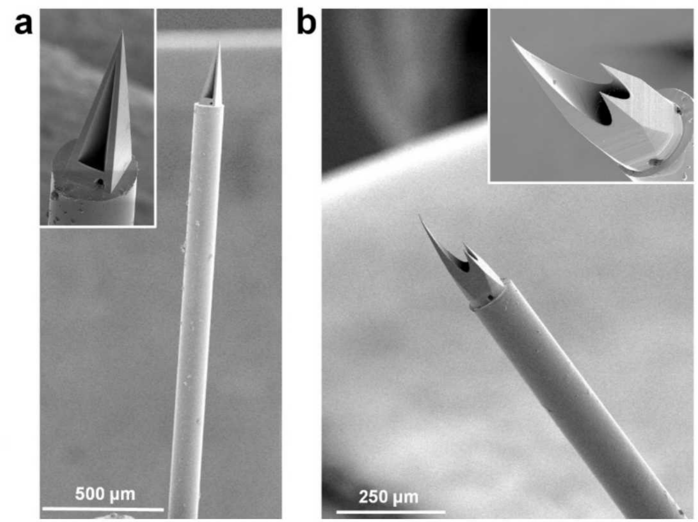
Spectroscopic interrogation provides increased utility of MN sensors for continuous real time monitoring of proteins and other biomolecules that are not easily detected eletcrochemically

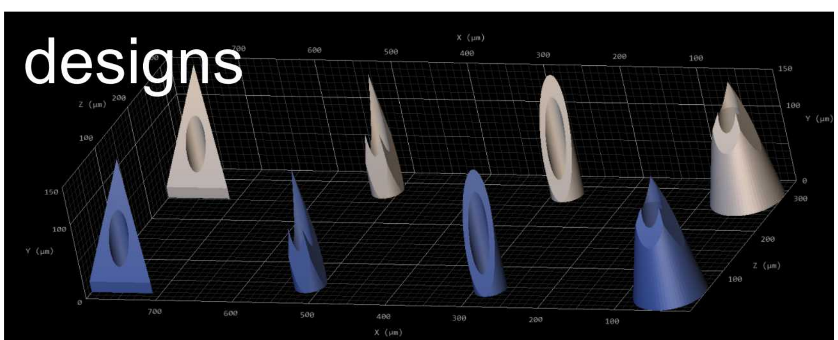


1. Core: 125 μm diameter
2. Cladding:
3. Buffer:
4. Jacket: ~700 μm dia.



1) Fix onto fiber
   a) 1.25 mm ceramic end
   b) SMA 950 standard connector
2) Fiber diameter compatible with NIR for label free Raman sensing.





Bryan Kaehr, Michael Gallegos, Samantha Martino

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Human and Animal Studies in Progress

Human studies in progress:

- Rigorous exercise in extreme environments
  - Grand Canyon R2R
- Pre- and post-vaccination

Animal studies in progress:

- Hypoxia
- Chem agent exposure

Grand Canyon Rim-2-Rim Hike

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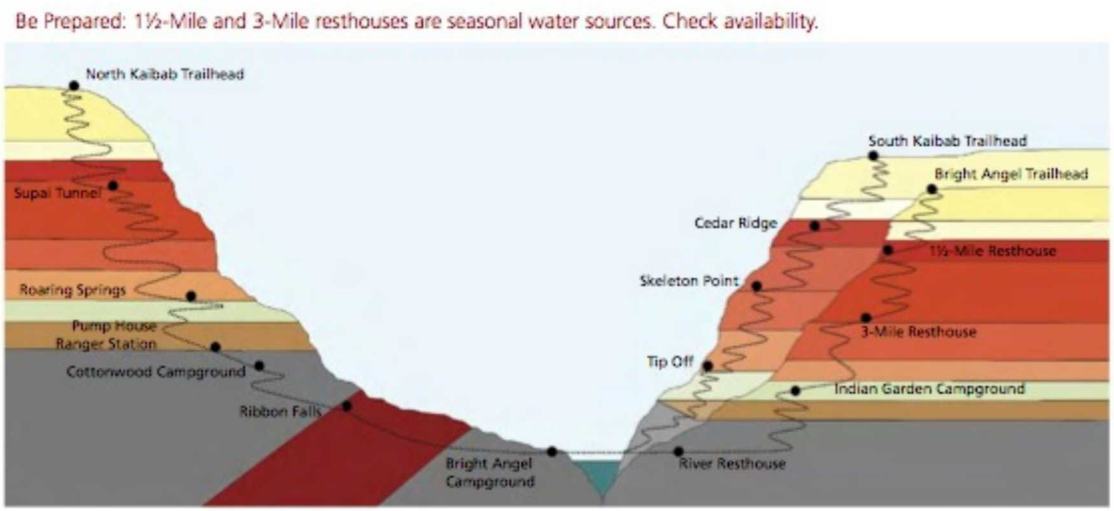
- Altitude and Temperature Change
- Extreme Environment
- Physical Strain
- 14.3 miles, 6,000 feet to the bottom
- 9.6 miles, 4,500 feet back to the South Rim

Figure 1. Signage at Bright Angel Trailhead, October 2015.



Source: https://www.nps.gov/grca/index.htm

Purpose of this Study

1) **Markers for Health**
   identify physiological, cognitive markers most related to health and task performance

2) **Wearable Devices**
   identify which COTS wearable devices are best for measurement and in rugged environments

3) **Health Events**
   use statistical analyses on collected data to identify which markers are most predictive of benign vs. traumatic health events

Grand Canyon R2R Study Design

- Subjects hike Grand Canyon wearing various wearable fitness devices that monitor physical activity
  - Heart rate
  - Heart rate variability
  - Breathing rate
  - GPS position
  - Cadence
  - Elevation
  - Temperature (ambient + skin)
  - Relative humidity
- Subjects take cognitive tests intermittently during hike
- Metabolic panel + demographic data collected before and after hike
- Two cohorts
  - Civilian volunteers
  - Military personnel

Collecting Physiological and Cognitive Data

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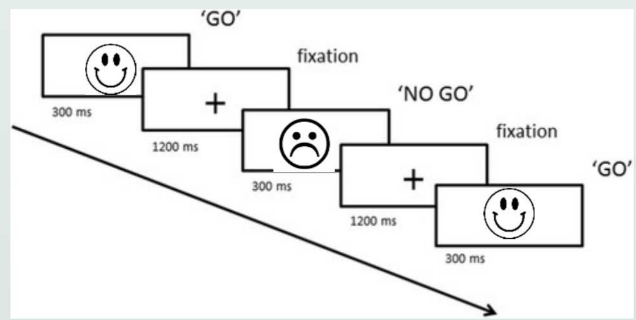
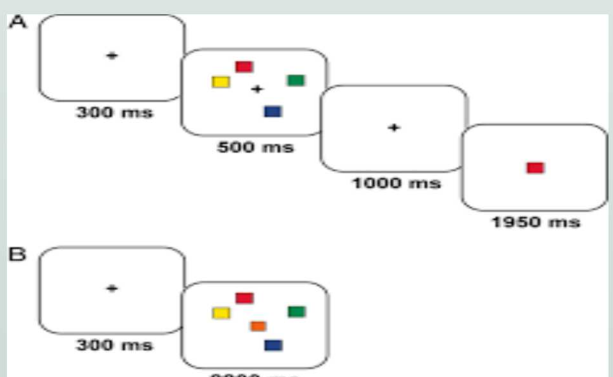
Package	Cognitve Tests	GPS	Elevation	Heart rate (ECG)	Heart rate (Wrist)	Heart rate (Forehead)	Cadence (Wrist)	Cadence (Torso)	Temperature (Ambient)	Temperature (Direct Sun)	Temperature (Skin)	Humidity	Total	Qty
Advanced 1	iPod Touch 6	Fenix 3 HR	Fenix 3 HR	Wahoo TickrX	Fenix 3 HR	LifeBeam SmartHat	Fenix 3 HR	Wahoo TickrX	SensorPush	SensorPush	Tempe	SensorPush	**$1,115**	10
Advanced 2	iPod Touch 6	Spartan Ultra	Spartan Ultra	Smart Sensor	(None)	LifeBeam SmartHat	Spartan Ultra	(None)	SensorPush	SensorPush	(None)	SensorPush	**$1,165**	10
Basic 1	iPod Touch 6	Vivoactive HR	Vivoactive HR	(None)	Vivoactive HR	(None)	Vivoactive HR	(None)	SensorPush	(None)	(None)	SensorPush	**$515**	35
Basic 2	iPod Touch 6	eTrex 10 + 2AA	(None - 'floors')	(None)	Fitbit Charge HR	(None)	Fitbit Charge HR	(None)	SensorPush	(None)	(None)	SensorPush	**$485**	15

Collecting Physiological and Cognitive Data

Flanker

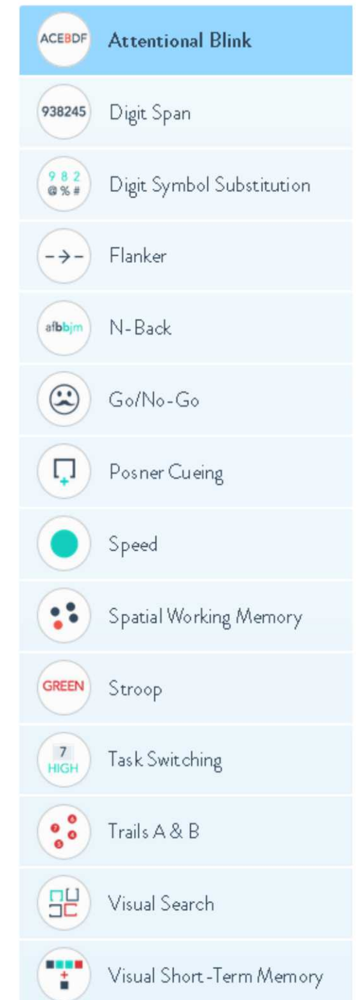
Go/No-go





VSTM

Brain Baseline



Cognitive data sample

First Test		Military	Civilian
Go/No-go:	Time	527 ms (sd = 66)	554 ms (sd = 87)
	Accuracy	0.964 (sd = 0.061)	0.957 (sd = 0.07[illegible])
Flanker:	Time	594 ms (sd = 109)	592 ms (sd = 98)
	Accuracy	0.935 (sd = 0.140)	0.943 (sd = 0.107)
VSTM:	Time	1012 ms (sd = 206)	1139 ms (sd = 309)
	Accuracy	0.779 (sd = 0.082)	0.732 (sd = 0.093)
Last Test		**Military**	**Civilian**
Go/No-go:	Time	488 ms (sd = 92)	530 ms (sd = 71)
	Accuracy	0.940 (sd = 0.060)	0.949 (0.070)
Flanker:	Time	496 ms (sd = 64)	559 ms (sd = 71)
	Accuracy	0.975 (sd = 0.026)	0.956 (sd = 0.098)
VSTM:	Time	978 ms (sd = 411)	1088 (sd = 343)
	Accuracy	0.779 (sd = 0.082)	0.702 (sd = 0.106)

Military performed better in 10 / 12 measures

Military's advantage increased in later trials, especially with response time: Could be less fatigue or better learning

Hypothesis: heart rate responds more quickly to changes in workload as fatigue sets in

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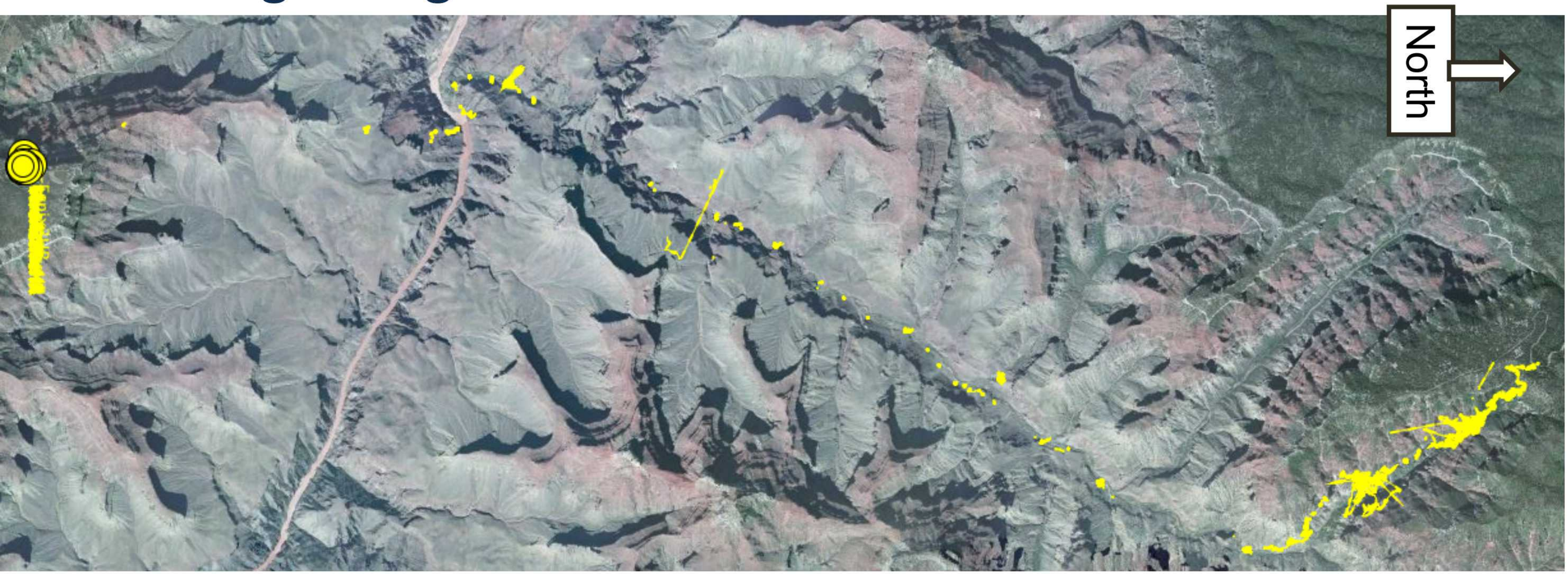
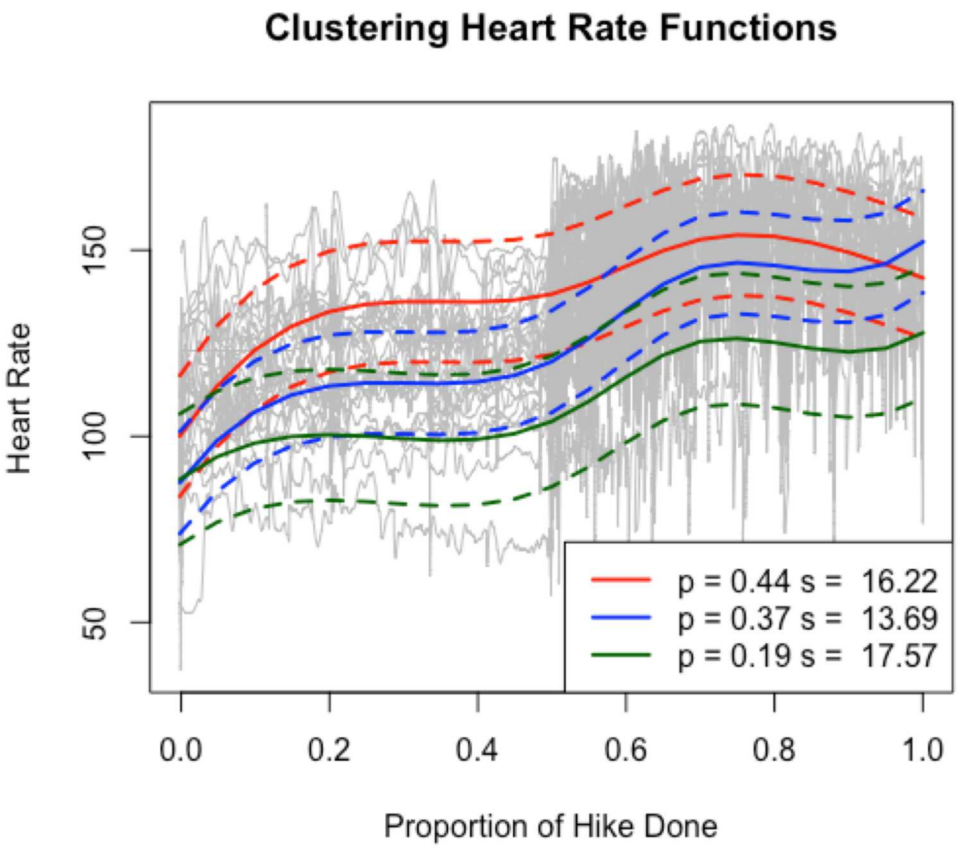
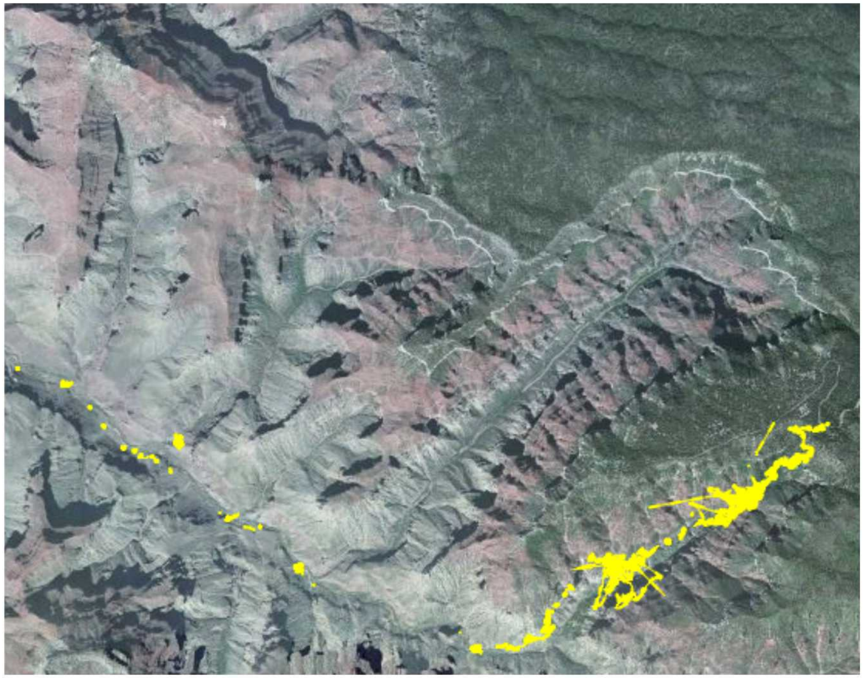
Detecting changes in workload - Breaks



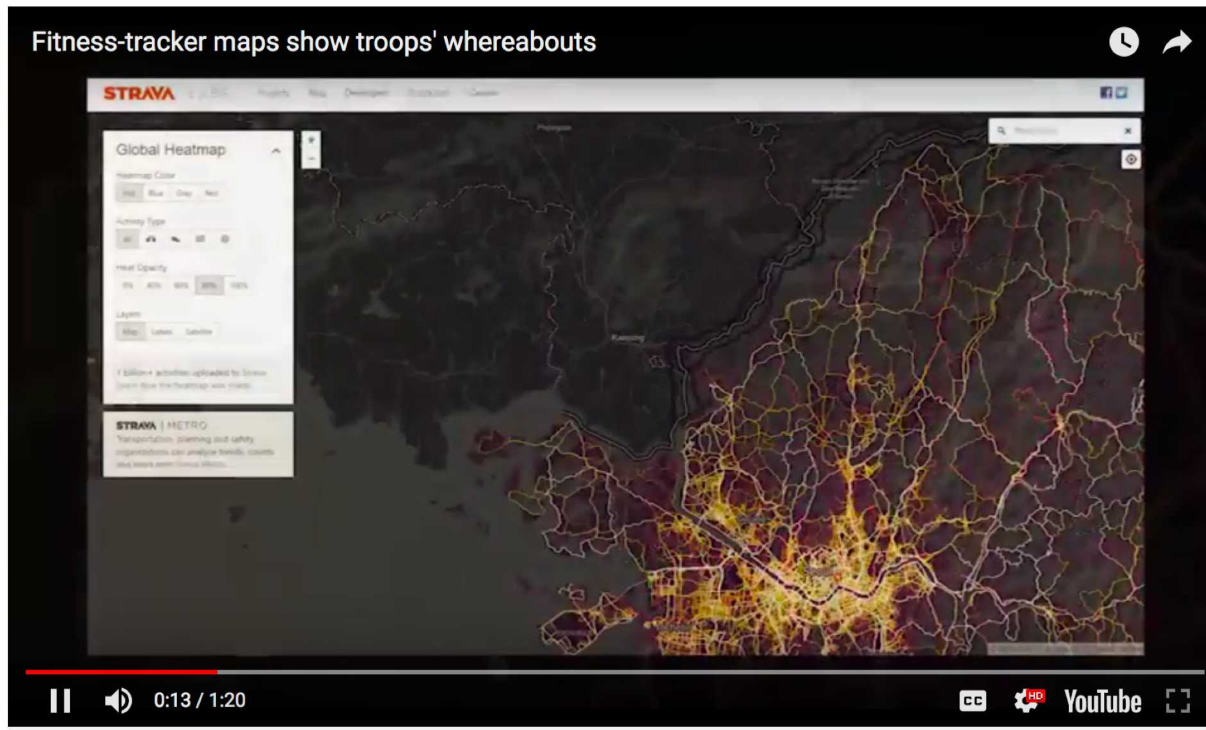
- This is everywhere that a subject's pedometer (wrist-based accelerometer) read 0 for at least 80% of samples in any 5-minute period
- GPS was not used to detect breaks, only indicate where they were at the time

Hypothesis: heart rate responds more quickly to changes in workload as fatigue sets in





Wearable tech could be a challenge for US military, experts say



Military cross-domain situational awareness is a "why-Sandia" arena; we provide sensors, data analysis and integration, actionable information, data security, and secure communications

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Partnerships essential to our success

- Partners have complementary expertise, capabilities, and sponsor relationships
- National labs and federal lab resources: LLNL, LANL, LBL, PNNL, USAMRIID, ECBC, CDC
- Universities: UCB, UC Davis, UCLA, UNM
- Industry: CRADAs and IP license agreements develop commercial sources for Sandia technology