MW CTR-IN Program 2023 annual meeting in Las Vegas
“Advancing Health Equity: From Local to Global”

2022-2023 CTR-IN Program **Multi Site Pilot Project** (NIGMS grant U54 GM104944)

Novel methods of assessing household wood smoke exposure in the rural Mountain West

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Mountain West CTR-IN Multi-site study
(Funding period: Jan 2022 – June 2023)

Novel methods of assessing household wood smoke exposure in the rural Mountain West

Aim 1: To test the responsiveness of **Household Exposure to Wood Smoke (HEWS)** to changes in wood smoke (WS) exposure by comparing scores between shoulder and peak heating seasons and further establish the validity of the HEWS by assessing the changes of scores with the changes in **Macrophage carbon Load (MaCL)** levels between seasons

Aim 2: To develop an **artificial intelligence-based algorithm** for quantifying MaCL levels that is high-throughput, scorer-independent, precise, and applicable in large-scale epidemiological studies

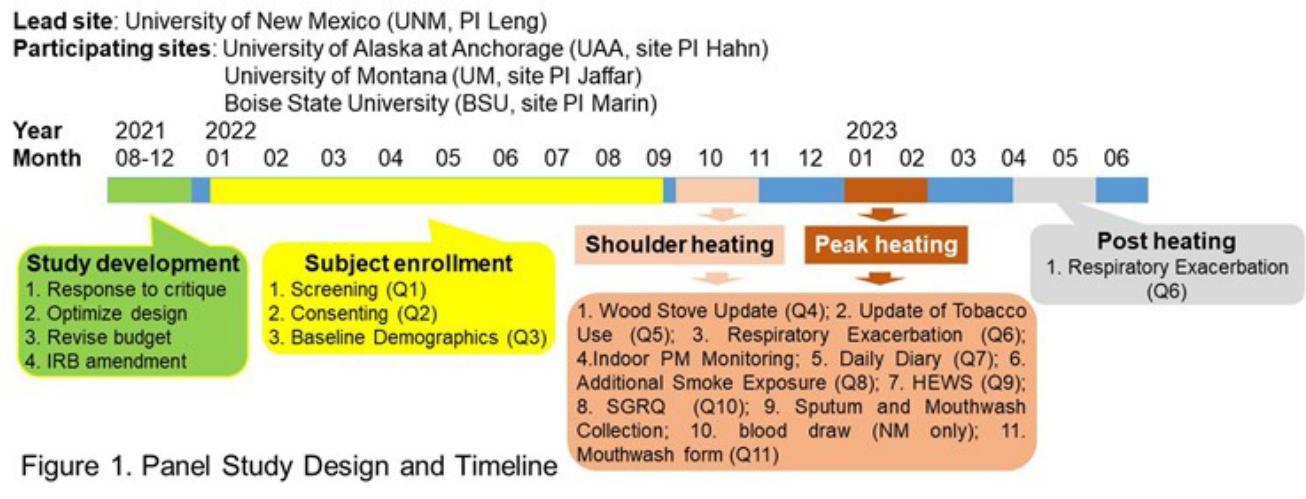
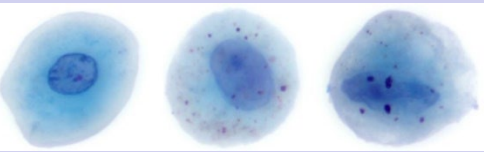


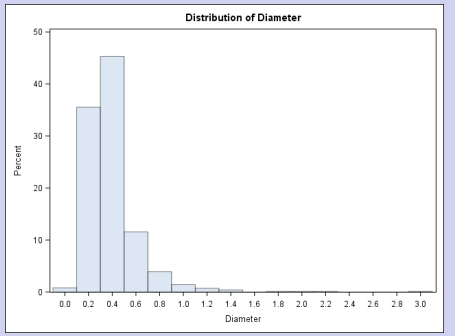
Figure 1. Panel Study Design and Timeline

Subject enrollment: use household wood stoves as the primary method for winter heating, 50-78 years old, and with and without lung comorbidities

1) During the past week how many hours was wood burned in the house over a 24 hours?
2) During the last week how often did you burn wood in your house?
3) Over the past week when wood was burning in the stove/fireplace I could smell smoke in the house?
4) Over the past week was wood burning in the stove/ fireplace while you sleep?
5) When the wood is burning it is your job to look after the stove/fireplace?
6) Over the past week when wood was burning in the stove/fireplace there was some smoke in the room?
7) When the wood is burning how close are you to the stove/fireplace?
8) Usually when wood was burning in the stove/fireplace I was in the same room?
9) Over the past week when you had wood burning in the stove/fireplace the door/front of the stove/fireplace was open?
10) Over the past week when you had wood burning in the stove/fireplace were the windows in the room open?
11) On average over the past week how many hours were you in the room where wood was burning to heat your house?
12) Typically it is your job to start the wood fire in the stove/fireplace?

Household Exposure to Wood Smoke





Macrophage Carbon Load

Expected outcomes:
The major yield will be a **validated HEWS** and an **AI-counting algorithm** for MaCL assay which collectively quantify individual exposure to indoor WS with different time frames (days for HEWS versus weeks for MaCL). These methods can be readily integrated into existing and future large-scale cohort studies addressing adverse health effects of WS exposure in the US.

Addition of a biomarker component to understand cancer risk of wood smoke exposure (at UNM study site only)

- Group 2A human carcinogen
- Limited cancer risk evidence in humans
- Abundance of human carcinogens (e.g., polycyclic aromatic hydrocarbons) in gaseous and particulate phases of wood smoke
- Mutagenicity and carcinogenicity of wood smoke extract in both in vitro and preclinical models
- Enroll subjects who are younger (40-69 years old) and have no severe comorbidities
- Collaboration with Dr. Lan and Rothman from NCI

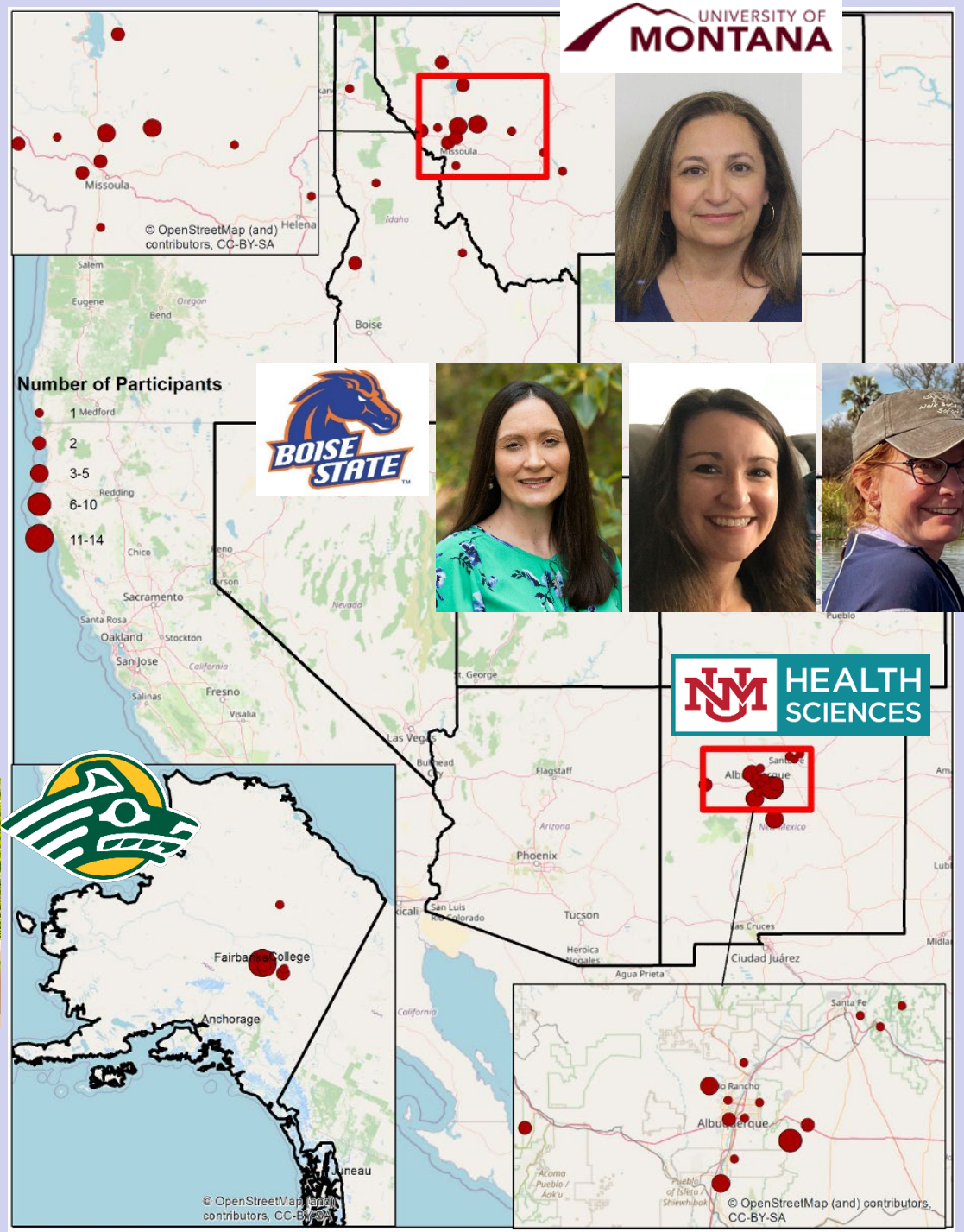
Table 2. Collection items for the Biomarker study

Item	Wood smoke exposure			Non-wood smoke exposure
	Summer	Shoulder heating	Peak heating	Peak heating
Month	Jul-Aug-Sep	Early Nov	Dec-Jan-Feb	Dec-Jan-Feb
n	50	A subset of 50*	50	25
Questionnaire				
Q4 Stove update		X	X	X
Q5 Tobacco use update	X	X	X	X
Q7 Daily diary		X	X	
Q8 Additional smoke exposure	X	X	X	X
Q9 HEWS		X	X	
Q10 SGRQ	X	X	X	X
Q11 Mouthwash form	X	X	X	X
Bio-specimen				
Sputum	X	X	X	X
Mouthwash	X	X	X	X
Blood	X		X	X
First-void urine	X	X	X	X
Nasal brush	X		X	X
Buccal scrape	X		X	X
Indoor PM monitor	X	X	X	X
Incentives	$100	$50	$100	$100

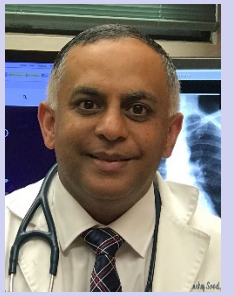
*An invited sub-group with age 50 to 60 years old

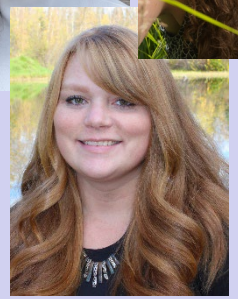
Alaska and Idaho flip the design by doing peak heating first
While NM and Montana maintain the original design

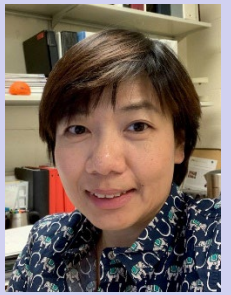
Research Team



Senior faculty

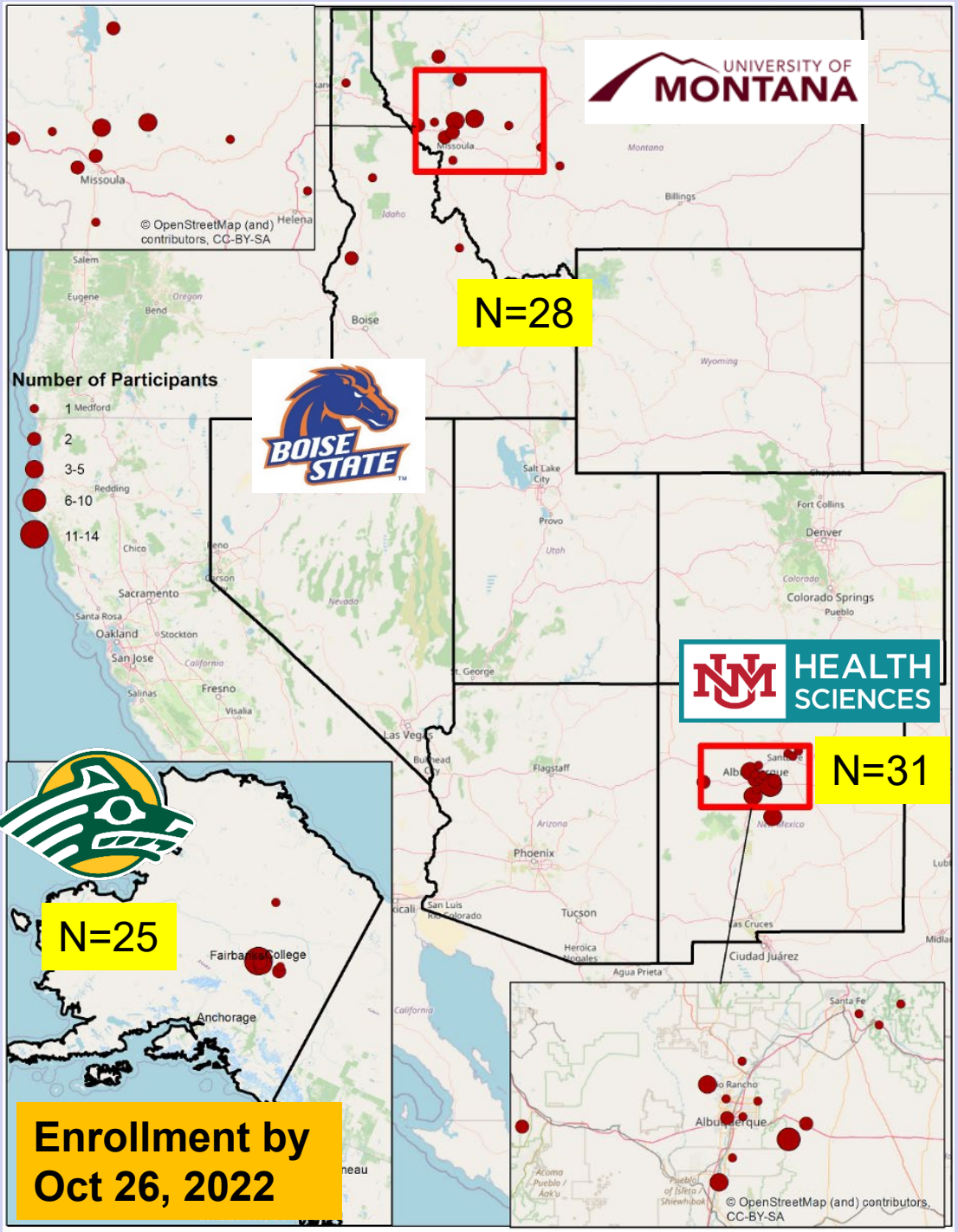






Subject enrollment

- Craigslist
- Community outreach
- Radio stations
- Homeowner associations
- UNM cancer center media
- Facebook ad (most effective)
- Independent newspaper
- Enroll household members
- Referral program



Characteristics by enrollment sites

	New Mexico	Idaho/Montana	Alaska
N	31	28	25
Age (n, %)			
Under 50 years	6, 19.4%	0, 0%	1, 4%
50 – 64 years	12, 38.7%	19, 67.9%	15, 60%
65 years and above	13, 41.9%	9, 32.1%	9, 36%
Non-Hispanic white (n, %)	24, 85.7%	26, 92.9%	25, 100%
Female sex (n, %)	15, 53.6%	16, 57.1%	13, 65%
College education (n, %)	19, 67.9%	16, 57.1%	19, 95%
Currently married (n, %)	22, 78.6%	14, 50%	15, 75%
Ever smokers (n, %)	9, 29.0%	7, 25%	5, 20%
Household annual income			
Less than $40K	9, 34.6%	9, 50%	2, 9.1%
$40K to $90K	5, 19.2%	3, 16.7%	5, 22.7%
More than $90K	9, 34.6%	6, 33.3%	10, 45.5%
Declined to answer	3, 11.5%	0, 0%	5, 22.7%
Exceed or meet the expenses (n, %)	16, 72.8%	9, 52.9%	14, 82.3%
Type of stove			
Standing alone stove (n, %)	18, 78.3%	17, 94.4%	17, 100%
Fireplace insert (n, %)	5, 21.7%	1, 5.6%	0, 0%
EPA certified (n, %)			
Yes	12, 52.2%	11, 61.1%	9, 56.3%
No	3, 13.0%	4, 22.2%	0, 0%
Do not know	8, 34.8%	3, 16.7%	7, 43.8%
Age of stove >10 years (n, %)	17, 73.9%	11, 61.1%	12, 70.6%
Neighborhood wood smoke smell			
Frequently or daily (n, %)	20, 71.4%	16, 57.2%	12, 60%

Community outreach

WOODSMOKE NEWSLETTER

WHAT IS AHEAD?

Shoulder-Heating Season $50

October – November 2022

Peak-Heating Season $100

December 2022 – February 2023

Summer Season $100

May – August 2023

WHAT IS THE PURPOSE OF THIS STUDY?

The purpose of the woodsmoke study is to assess how indoor wood burning affects air quality and health.

Commonly asked questions:

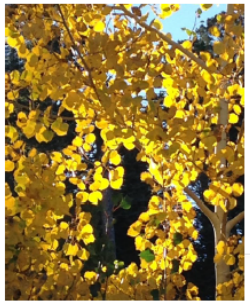
Here are two of our FAQs about the upcoming collection season.

Q: Which bio-specimen will I collect in the peak-heating season?
Self-Collected: sputum, mouthwash, and first-void urine
Collected by study team: blood draw, nasal, and buccal

Q: Is it important to connect the monitor to Wi-Fi?
It is very important to connect the air monitor to Wi-Fi to ensure that the air quality data is properly recording.
Note: If you do not have Wi-Fi, accommodations are available.

"Research is formalized curiosity. It is poking and prying with a purpose."

~ Zora Neale Hurston



FACTS ABOUT THE STUDY

How many sites are working on this study?
There are 4 sites: University of New Mexico, University of Montana, University of Alaska-Anchorage, and Boise State University in Idaho

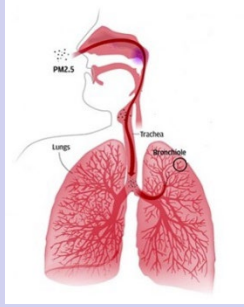
How many people are participating?
So far the wood smoke study has **82** participants! We are still enrolling people and we hope this number goes up.

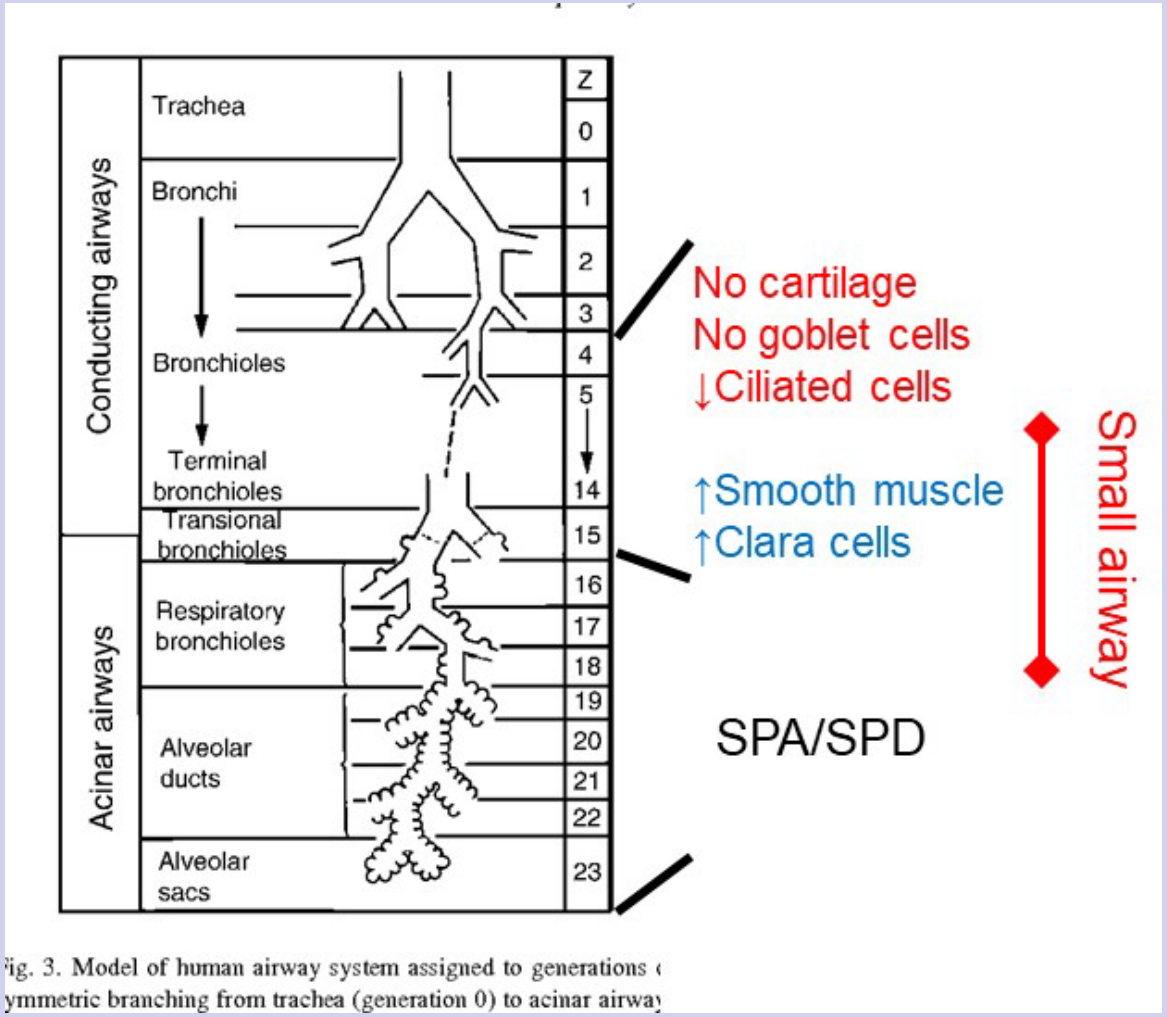
THANK YOU SINCERELY FOR BEING PART OF OUR PROJECT!

MW CTR-IN

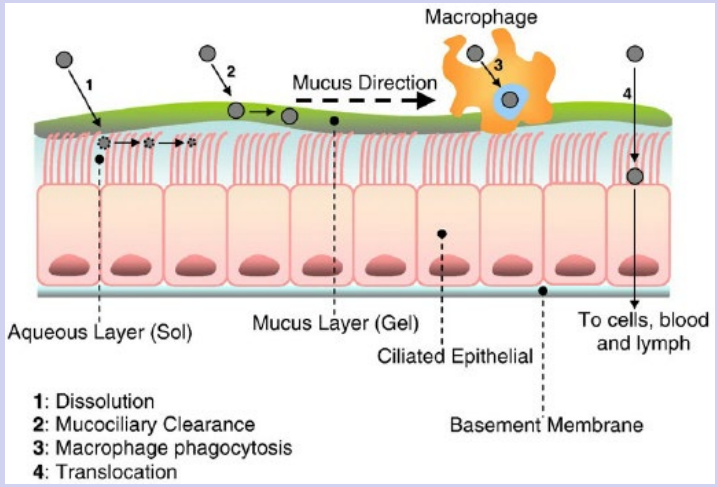
UNM COMPREHENSIVE CANCER CENTER

Mucociliary clearance and phagocytosis are two major mechanisms clearing inhaled combustion particles in the lungs

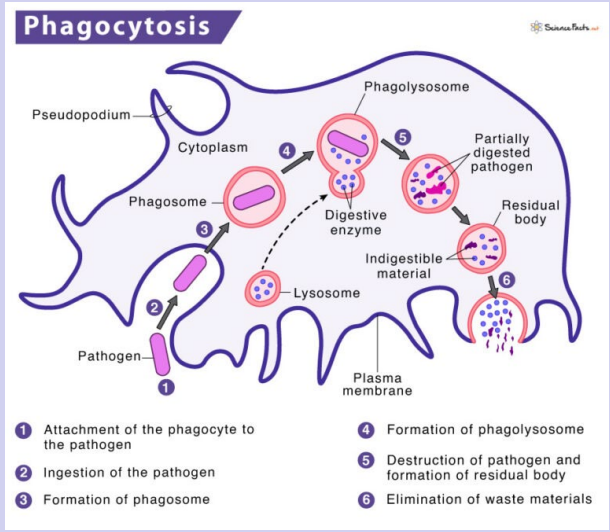




ig. 3. Model of human airway system assigned to generations
ymmetric branching from trachea (generation 0) to acinar airway

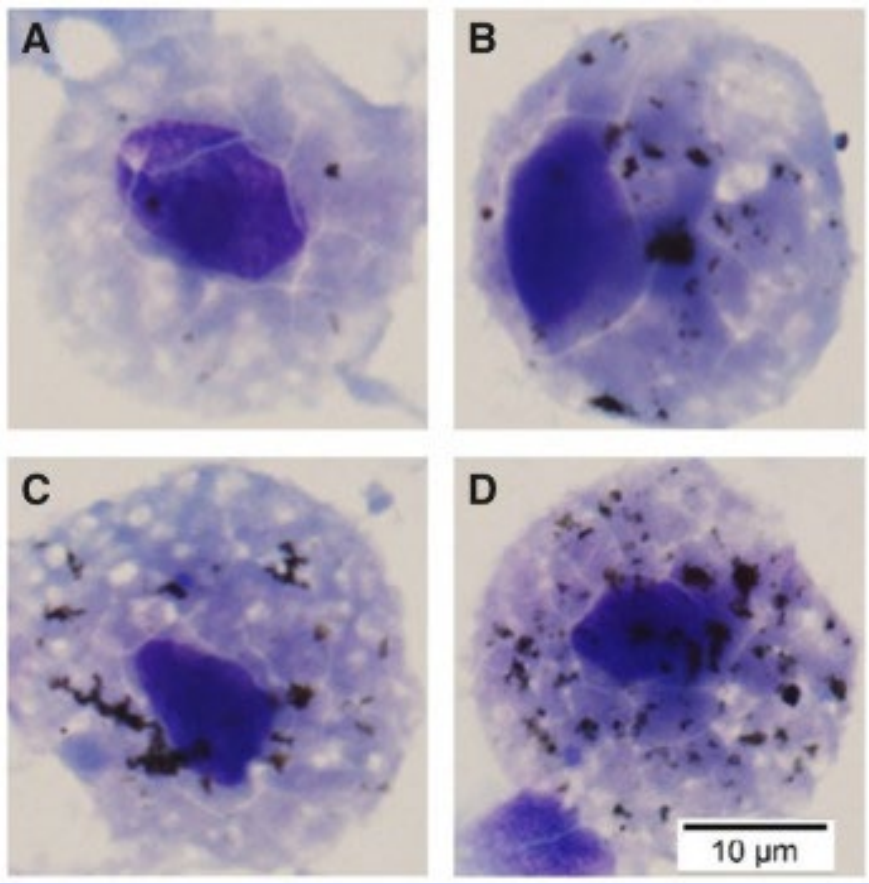


Mucociliary clearance



Phagocytosis

Macrophage carbon load is a lung dose biomarker for black carbon particles



Carbon black packers
Cao et al. Toxicological Sciences 2020

- Engulfed black carbon can be detected under light microscope as “black” particles with elemental carbon composition confirmed using spectrometry methods
- Provide a tool to assess lung dose from total environment exposure
- Associated with multiple pulmonary and extra-pulmonary outcomes
- Clearance of carbon particles in airway macrophages is a slow process (reduce 0.006-0.013 μm^2/day) and may take weeks to months to occur depending on peak exposure

Definition of episodic elevation of combustion emitted PM2.5

- Annual PM2.5 levels range from 5.2 μg/m^3 to 7.1 μg/m^3 in Albuquerque between 2001 and 2010.
- Based on EPA air quality monitor data, we identified seven periods with elevated PM2.5 levels (daily PM2.5 >10 μg/m^3) over extended period of time (2 wks or more) in Albuquerque.
  - Summer: wood smoke invasion from wild fires in surrounding counties or States.
  - Winter: local wood burning for heating
- We also identified three periods with low PM2.5 levels (30-day average PM2.5 prior to sputum collection <4 μg/m^3).

Seven episodes with elevation in ambient PM2.5 levels

Period	Number of day		Mean ± SD ($\mu g/m^3$)	Max daily ($\mu g/m^3$)	Sources
	All	≥10 $\mu g/m^3$			
24NOV2000 - 29JAN2001	67	40	11.9 ± 6.1	30.8	Local sources, e.g., heating
24JUN2002 - 07JUL2002	14	12	14.1 ± 7.0	33.6	Sitgreaves National Forest, West Malpais wilderness, Gila National Forest, Cottonwood Canyon
25NOV2002 - 16DEC2002	22	15	12.7 ± 5.2	19.8	Local sources, e.g., heating
03DEC2003 - 23JAN2004	52	16	9.8 ± 4.9	22.0	Local sources, e.g., heating
20MAY2004 - 13JUL2004	55	22	10.6 ± 6.4	46.8	Capitan Mountains, Gallinas mountains, Strayhorse, Chain of Craters Mesa, Diener, Indian Peaks, Three Forks, Grapevine Canyon, Turkey Ridge, Midnight Mesa, Gila National Forest, Tonto National Forest, Coconino National Forest, Pinaleno Mountains
29JUN2005 - 06AUG2005	39	15	10.0 ± 4.7	29.3	Canyon Creek Mountains, Tonto National Forest, New River Mountains, Black Peak
22NOV2006 - 31JAN2007	71	28	10.2 ± 6.6	35.6	Local sources, e.g., heating

Sputum slides collected 9-70 days post first day of episodic elevation were pulled with an average of 41 days.

Satellite data for fires between 13JUN2004 and 13JUL2004

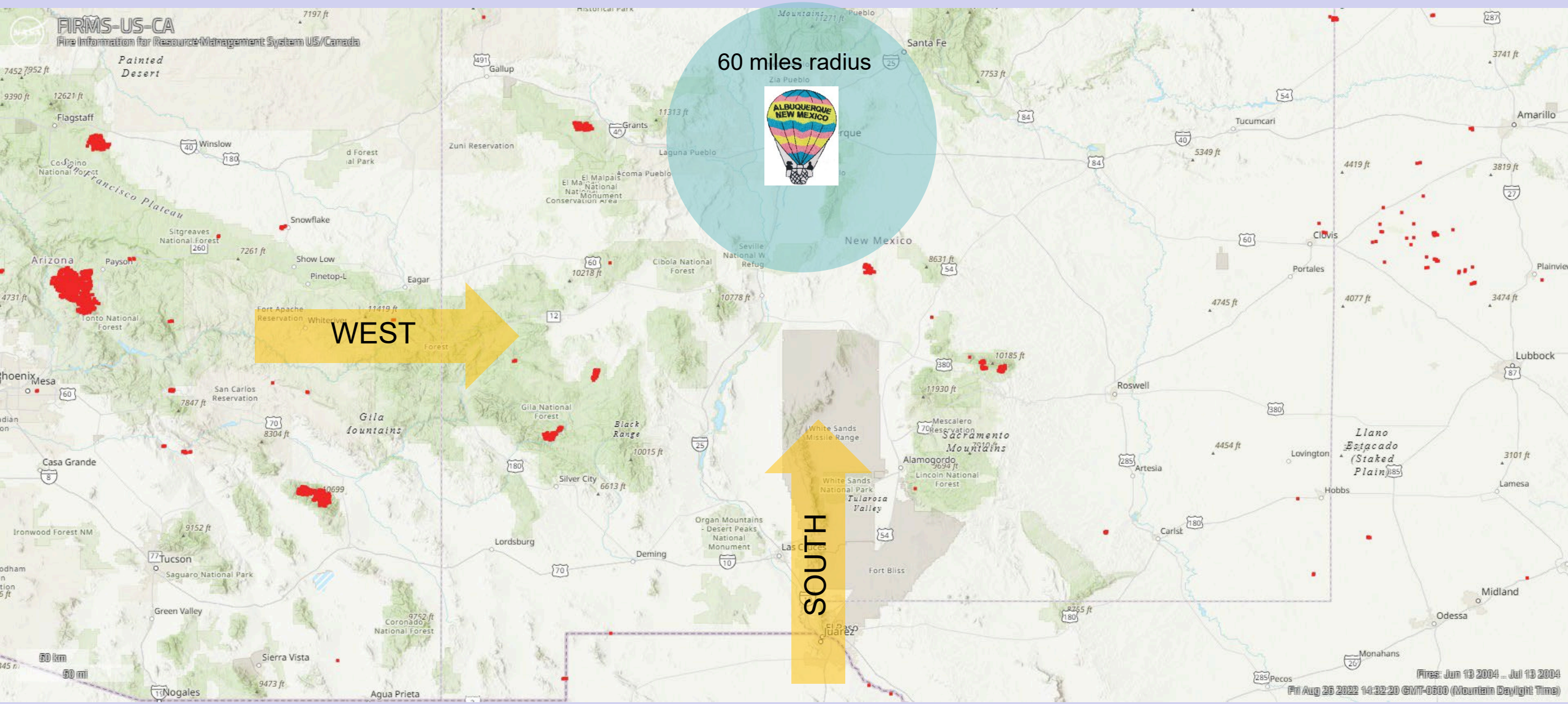
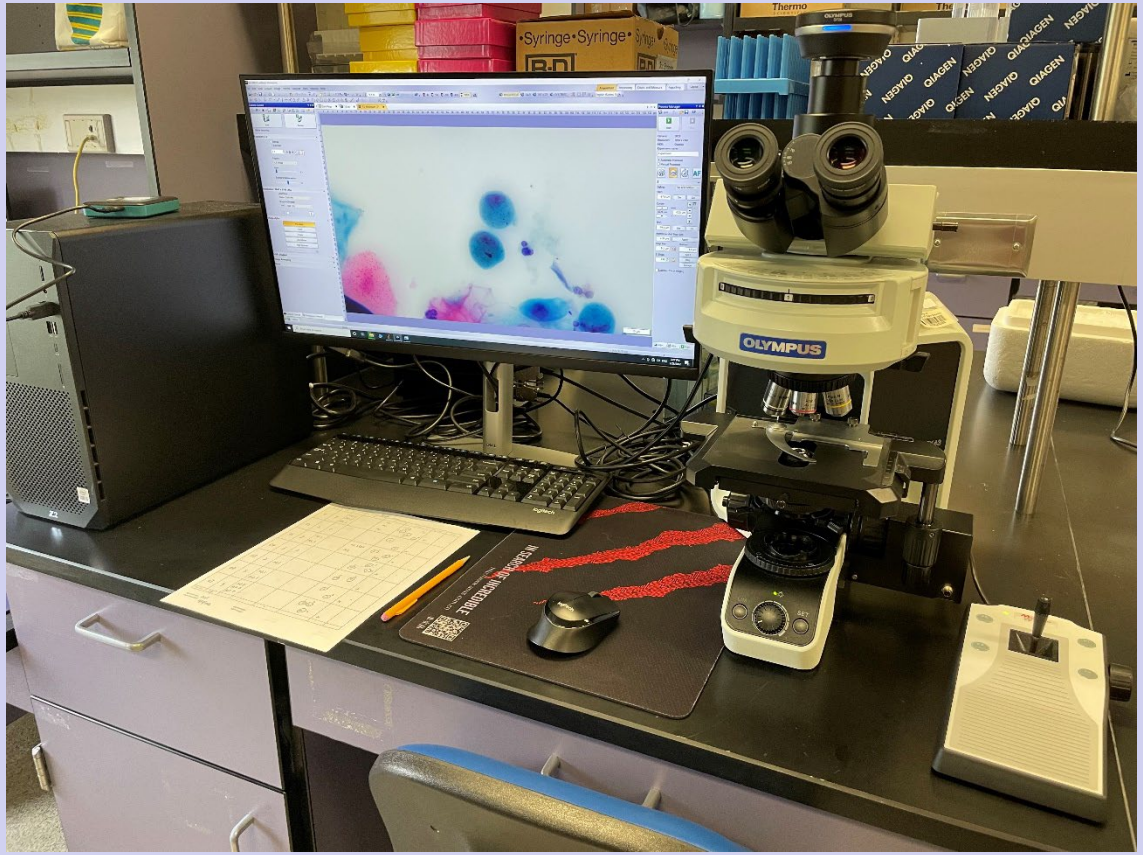


Table 1. Characteristics of the study subjects (n=88)

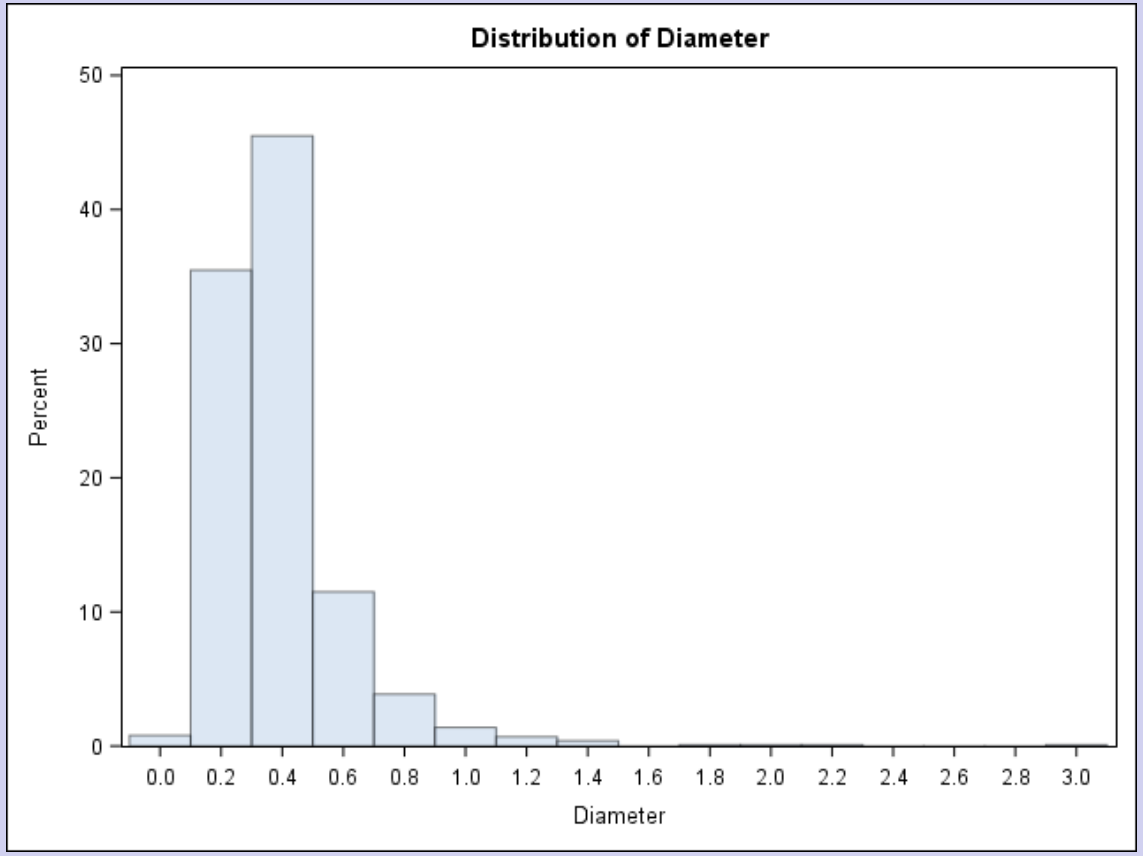
Variable	Value
n	88
Age (yr, mean ± SD)	55.3 ± 8.1
Male (n, %)	6, 6.8
Ethnicity	
Non-Hispanic white (n, %)	74, 84.1
Others (n, %)	14, 15.9
Current smoker (n, %)	58, 65.9
Packyears (mean ± SD)	42.9 ± 26.5
BMI (mean ± SD)	28.7 ± 6.2
BMI>25 (n, %)	61 (69.3)
Ever woodsmoke exposure (n, %)	21, 23.9
Plasma CC16 (n, ng/ml, mean ± SD)	48, 3.17 ± 1.64
MaCL measurements	
Area of macrophage (μm^2, mean ± SD)	182.3 ± 34.0
Number of particles per MA (median, Q1-Q3)	1, 1-2
Area of particles per MA (μm^2, median, Q1-Q3)	0.11, 0.06 - 0.21
% cell area occupied by carbons (%, median, Q1-Q3)	0.057, 0.032 - 0.115
% cells with particles (%, mean ± SD)	67.5 ± 15.5

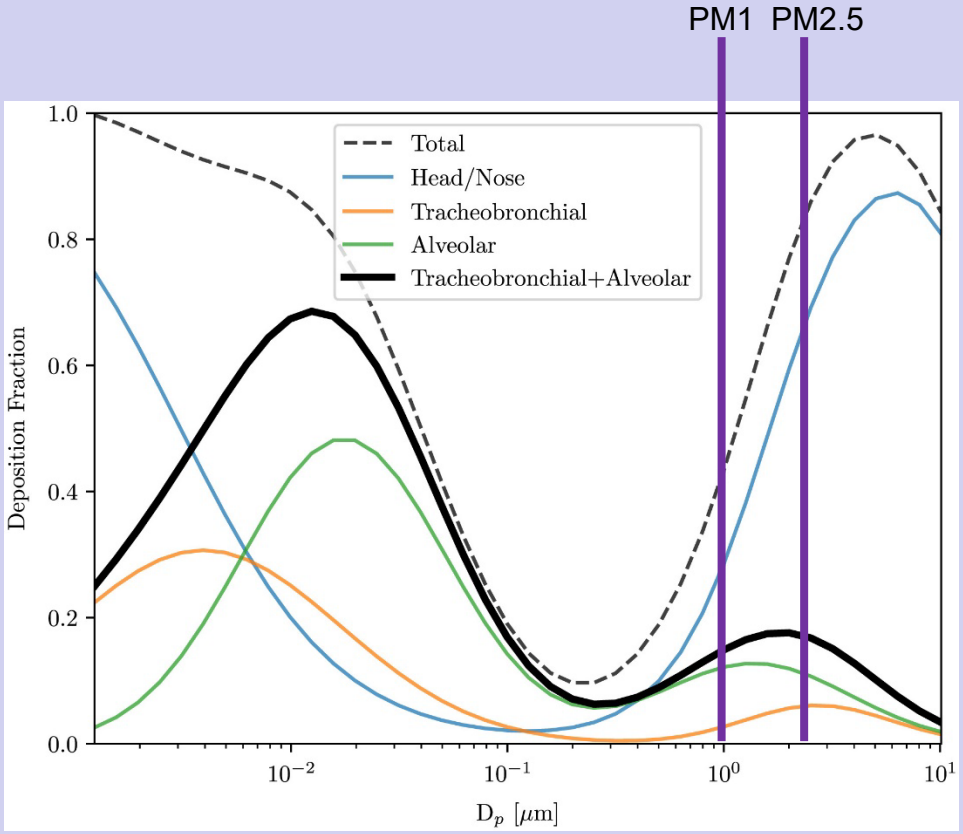
Image acquisition system



- Olympus BX43 mounted with a DP28 camera
- A 100× oil immersion lens
- A motorized Z drive
- Z-stack images with 100 nm as the depth interval to cover the entire cell depth
- A flattened image will be generated with most contrasted features at each depth projected
- 1 pixel = 34.5 nm

Size distribution of 1009 engulfed individual particles (μm)





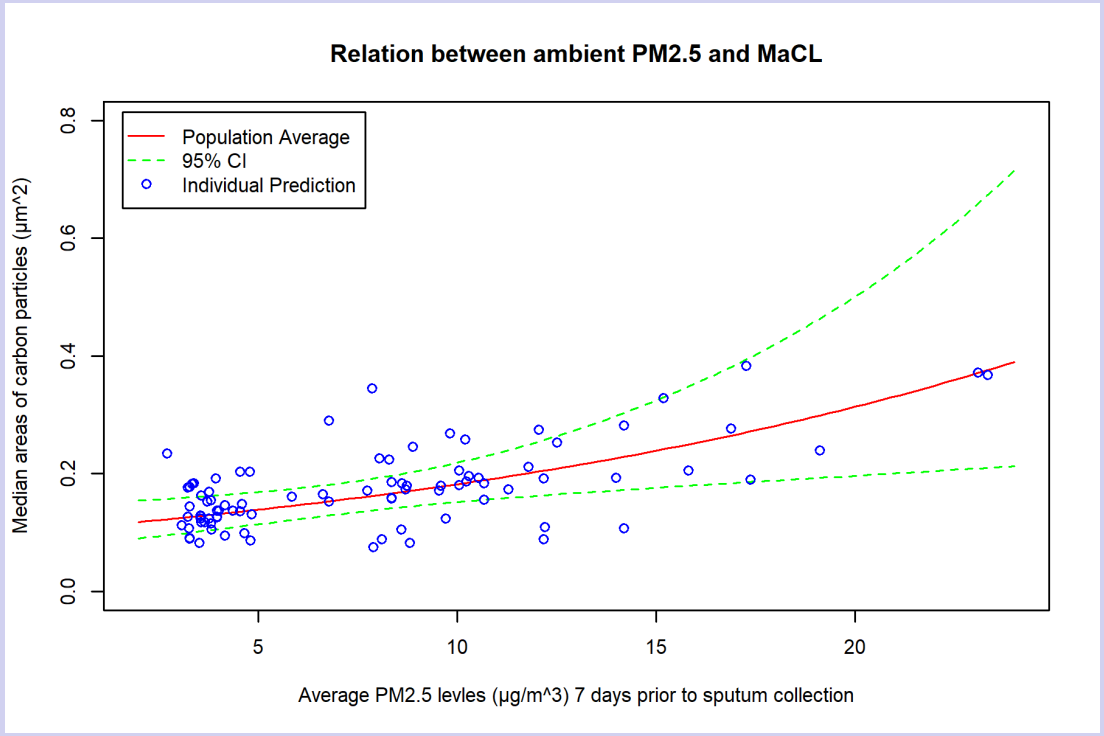
Size dependent deposition curves for PMs

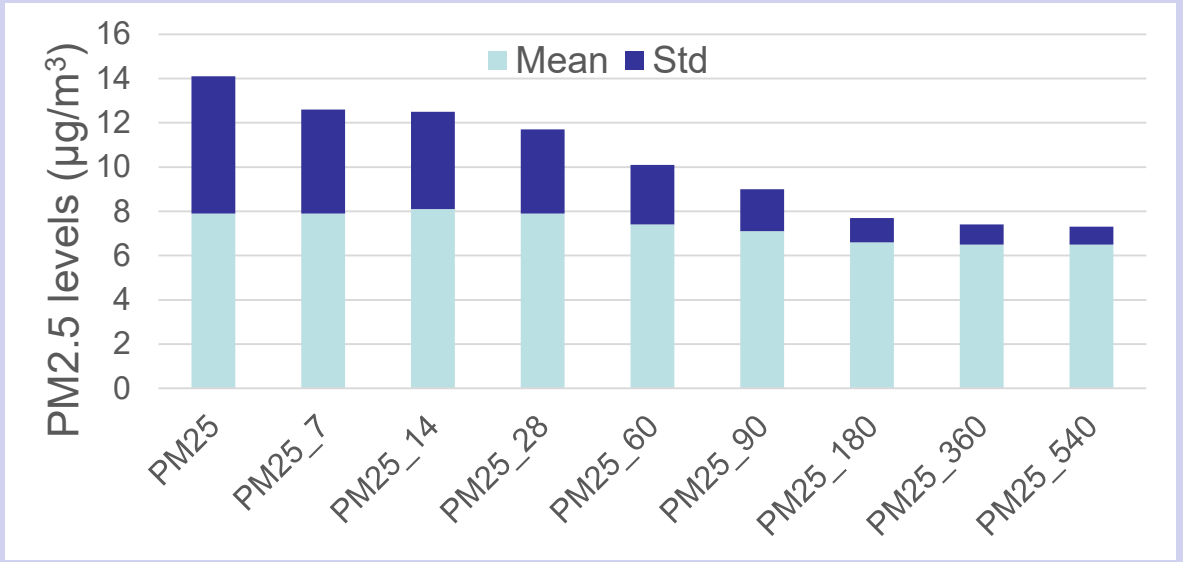
98% engulfed individual particles have diameters <1 μm

Kodros et al. GeoHealth. 2018

Table 2. Associations between ambient PM2.5 levels and MaCL endpoints

MaCL	Average PM2.5 level (per 5 μg/m^3 increase)								
	0d	7d	14d	28d	2m	3m	6m	12m	18m
NOP	1.23	1.48	1.46	1.41	1.59	1.96	3.68	4.53	6.87
	(1.11 - 1.37)	(1.28 - 1.71)	(1.21 - 1.77)	(1.11 - 1.77)	(1.15 - 2.21)	(1.23 - 3.11)	(1.56 - 8.72)	(1.70 – 12.11)	(1.99 – 23.77)
	0.0001	<0.0001	<0.0001	0.0041	0.0056	0.0045	0.003	0.0026	0.0023
AP	1.15	1.31	1.32	1.29	1.43	1.69	2.49	2.99	3.93
	(1.00 - 1.32)	(1.12 - 1.54)	(1.10 - 1.59)	(1.03 - 1.61)	(1.04 - 1.96)	(1.06 - 2.68)	(1.12 - 5.52)	(1.14 - 7.82)	(1.27 - 12.18)
	0.055	0.0009	0.003	0.024	0.026	0.027	0.025	0.026	0.018
%CWP	1.05	3.70	4.05	3.90	6.35	9.82	15.55	17.87	21.34
	1.35	1.75	1.90	2.15	3.00	4.30	7.73	9.11	10.85
	0.44	0.038	0.035	0.075	0.039	0.026	0.048	0.053	0.053

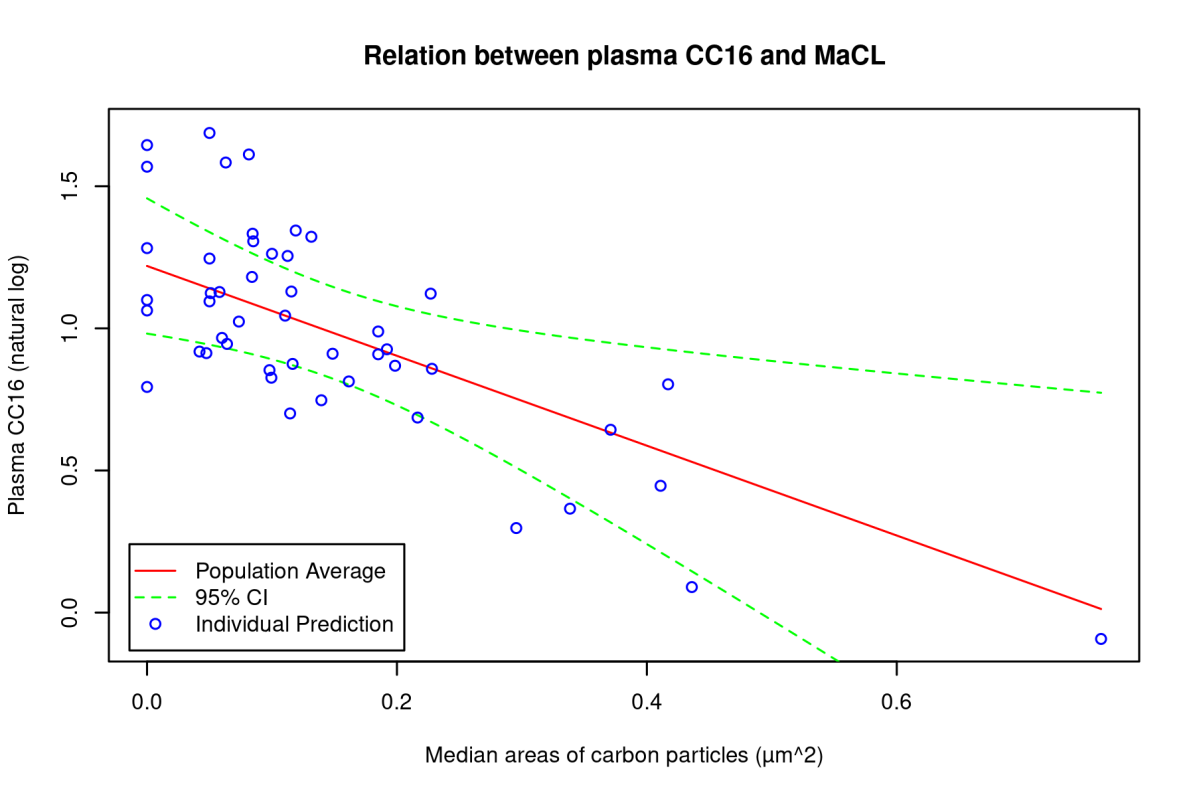




Standard deviation reduces for longer period

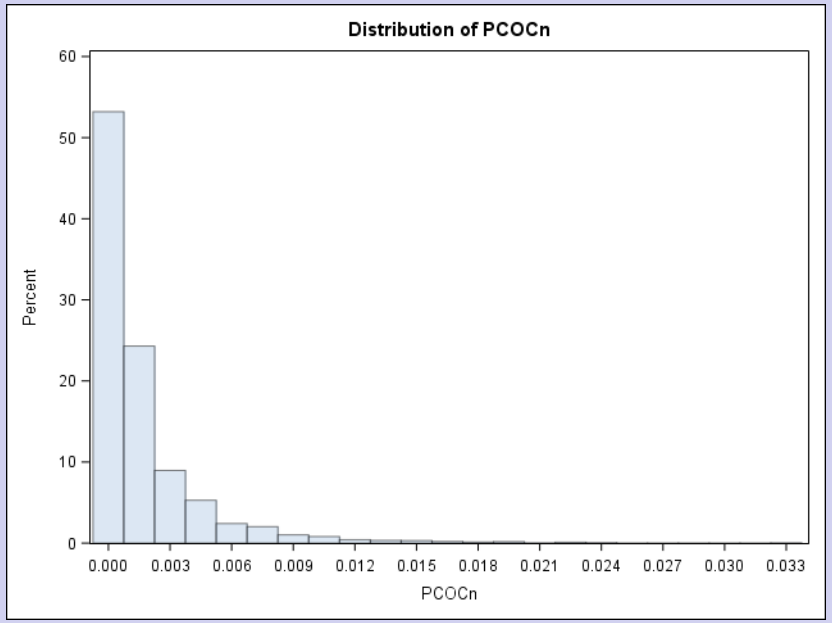
Table 4. Associations between MaCL levels and plasma CC16 (n=48)

MaCL variable	Unit	IQR	CC16 concentration ratio	P
NOP	count	1	0.84 (0.73 - 0.96)	0.011
AP	μm^2	0.134	0.81 (0.69 - 0.95)	0.011
%CWP	%	20.4	0.89 (0.70 - 1.13)	0.33



- MaCL was associated with lower CC16 in plasma, suggesting the injury of club cells
- Ambient PM2.5 at different time frames did not affect plasma CC16 levels, suggesting importance of considering lung dose.

Macrophages with high carbon load have more potent effects



PCOC = percentage of cell area occupied by carbon particles

N = 4429
Median = 0.064%
Q3 = 0.20%
95th = 0.72%

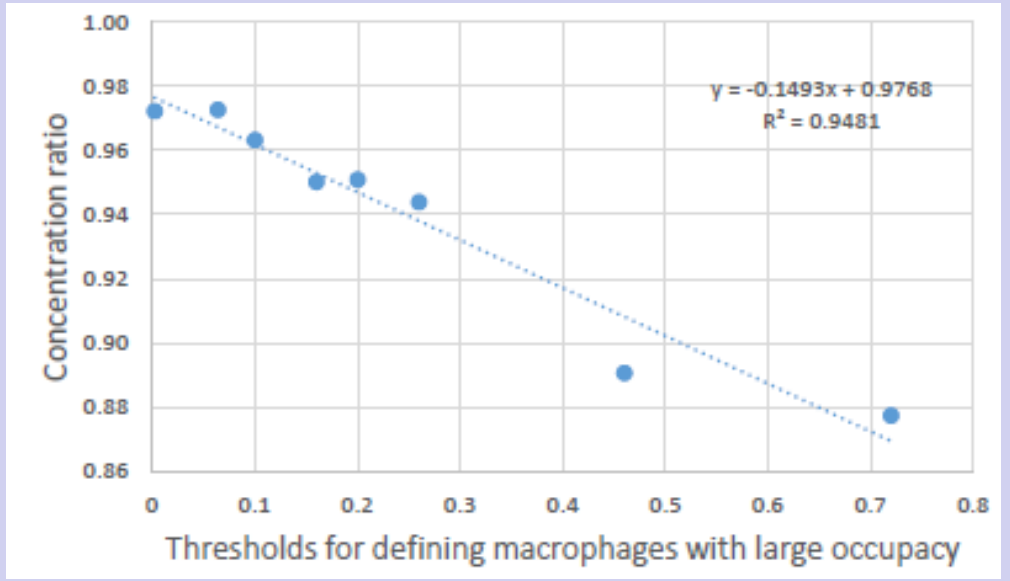
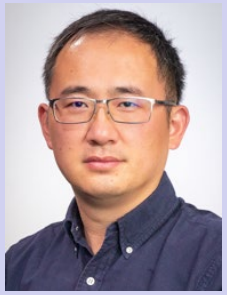
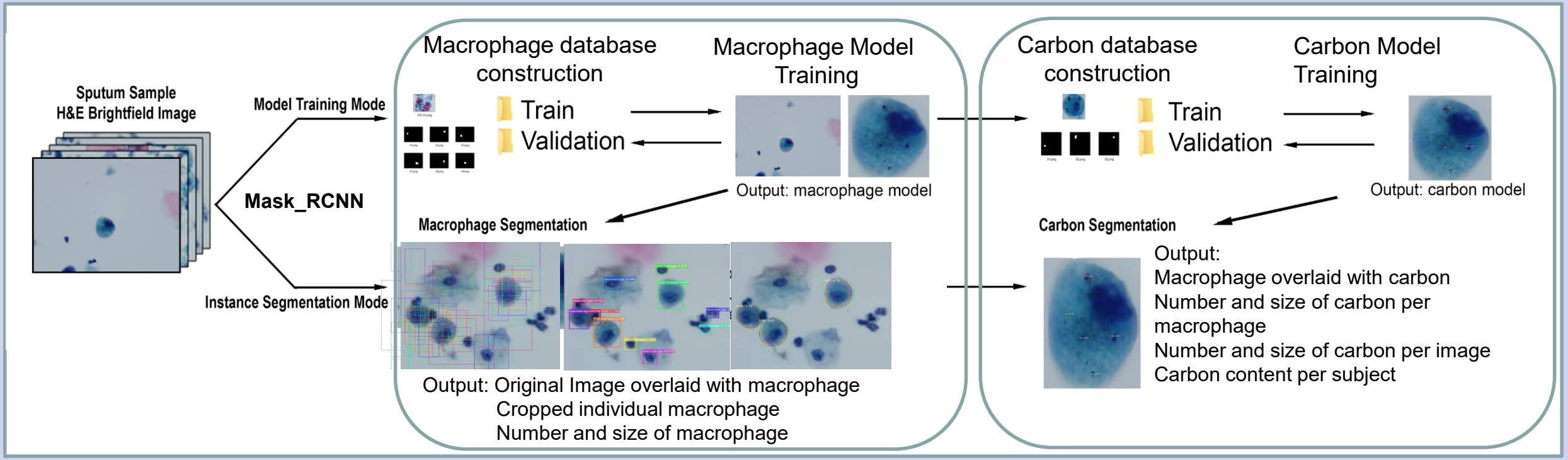


Table 5. %cell with higher carbon load and plasma CC16 (n=48)

PCOC threshold	Mean ± SD	CC16 concentration ratio	P
Minimal (0.0025%)	67.5 ± 15.5	0.97 (0.92 - 1.03)	0.33
Median (0.064%)	46.4 ± 21.2	0.97 (0.93 - 1.02)	0.22
60th percentile (0.10%)	37.1 ± 20.3	0.96 (0.92 - 1.01)	0.10
70th percentile (0.16%)	27.6 ± 18.1	0.95 (0.90 – 1.00)	0.046
75th percentile (0.20%)	23.3 ± 17.0	0.95 (0.90 – 1.00)	0.064
80th percentile (0.26%)	18.6 ± 15.2	0.94 (0.89 - 1.00)	0.059
90th percentile (0.46%)	9.4 ± 9.5	0.89 (0.81 - 0.98)	0.018
95th percentile (0.72%)	5.0 ± 5.5	0.88 (0.74 - 1.04)	0.13

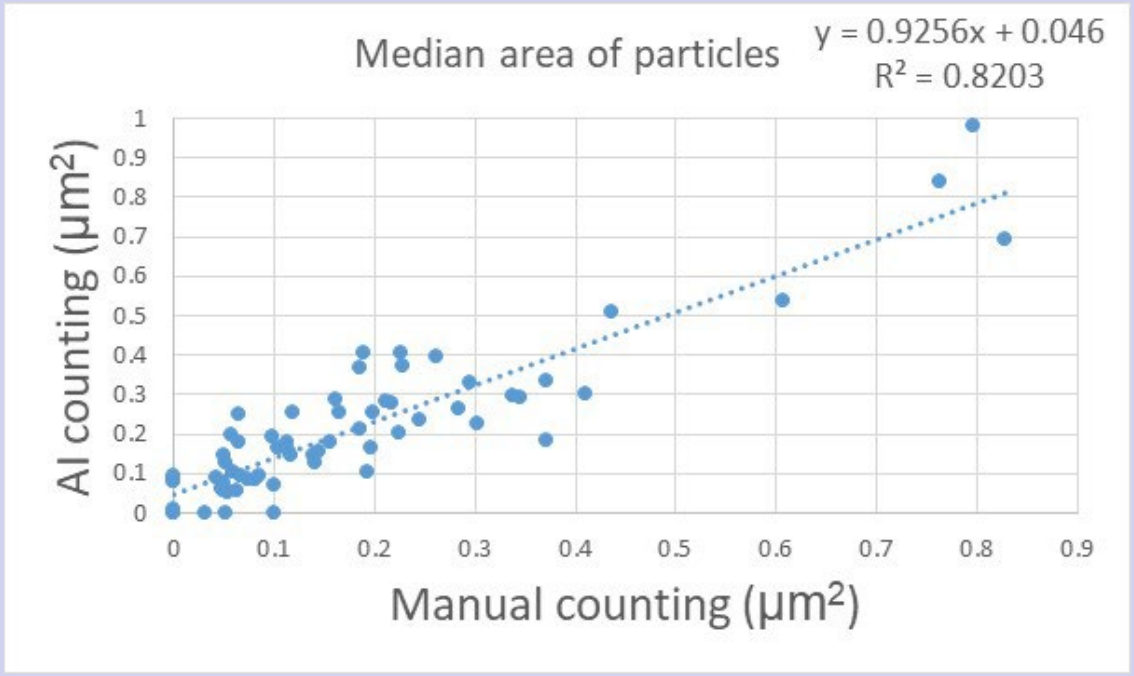


MacLEAP: Machine-Learning algorithm for Engulfed cArbon Particles

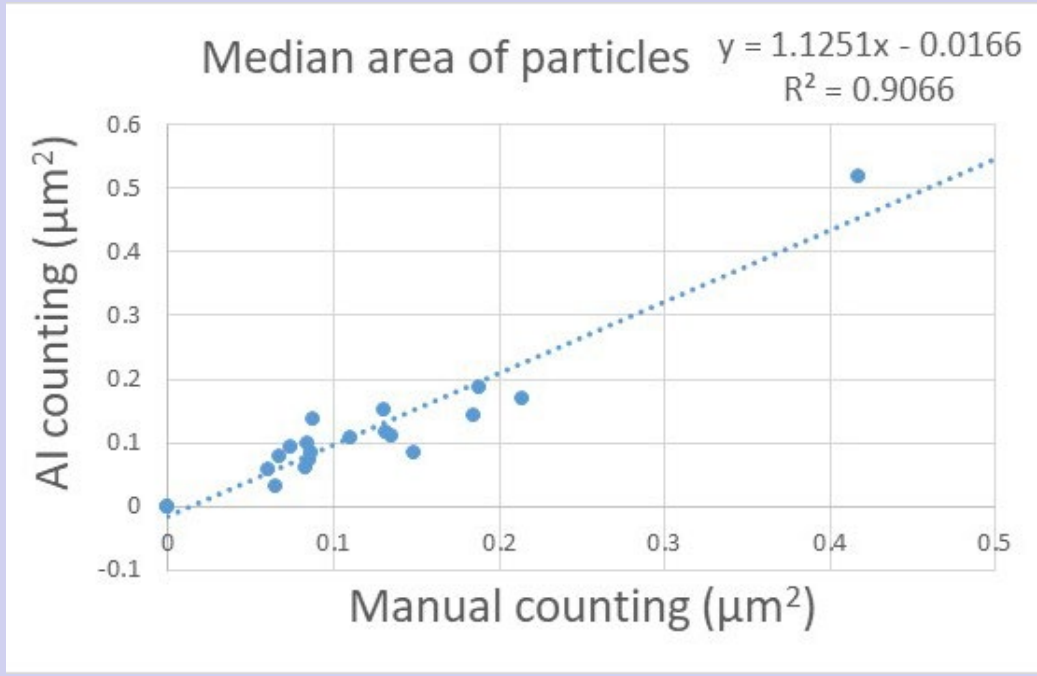


R-CNN: Region-Based Convolutional Neural Network

MacLEAP: Machine-Learning algorithm for Engulfed cArbon Particles



Algorithm development in 66 subjects



Algorithm Validation in 22 subjects

Excellent to outstanding correlations between manual scoring and AI counting

MacLEAP: Machine-Learning algorithm for Engulfed cArbon Particles

Table 6. Associations between ambient PM2.5 levels and MacLEAP MaCL endpoints

MaCL	Average PM2.5 level (per 5 µg/m^3 increase)								
	0d	7d	14d	28d	2m	3m	6m	12m	18m
NOP	1.13	1.32	1.33	1.27	1.38	1.57	2.19	2.50	4.00
	(1.02 - 1.26)	(1.14 - 1.52)	(1.11 - 1.58)	(1.03 - 1.57)	(1.03 - 2.41)	(1.03 - 2.41)	(1.00 - 4.79)	(1.01 - 6.20)	(1.31 - 12.24)
	0.021	0.0002	0.0018	0.026	0.034	0.036	0.049	0.048	0.015
AP	1.13	1.30	1.35	1.33	1.48	1.78	3.07	3.63	6.38
	(0.98 - 1.31)	(1.10 - 1.55)	(1.11 - 1.63)	(1.06 - 1.67)	(1.07 - 2.07)	(1.08 - 2.92)	(1.28 - 7.34)	(1.26 - 10.45)	(1.89 - 21.51)
	0.081	0.0024	0.0022	0.013	0.020	0.023	0.012	0.017	0.0028

Table 7. Associations between MaCL levels and plasma CC16 in 48 LSC subjects

MaCL variable	Counting method	IQR	CC16 concentration ratio	P
Number of particles (count)	Manual	1	0.84 (0.73 - 0.96)	0.011
	AI	1.75	0.76 (0.61 - 0.95)	0.018
Area of particles (µm^2)	Manual	0.134	0.81 (0.69 - 0.95)	0.011
	AI	0.195	0.78 (0.63 - 0.98)	0.030

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Respiratory Research

RESEARCH Open Access

Wood smoke exposure affects lung aging, quality of life, and all-cause mortality in New Mexican smokers

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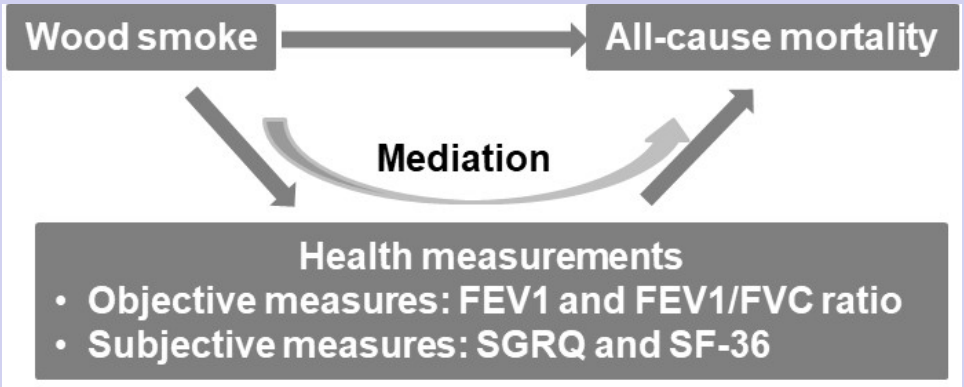
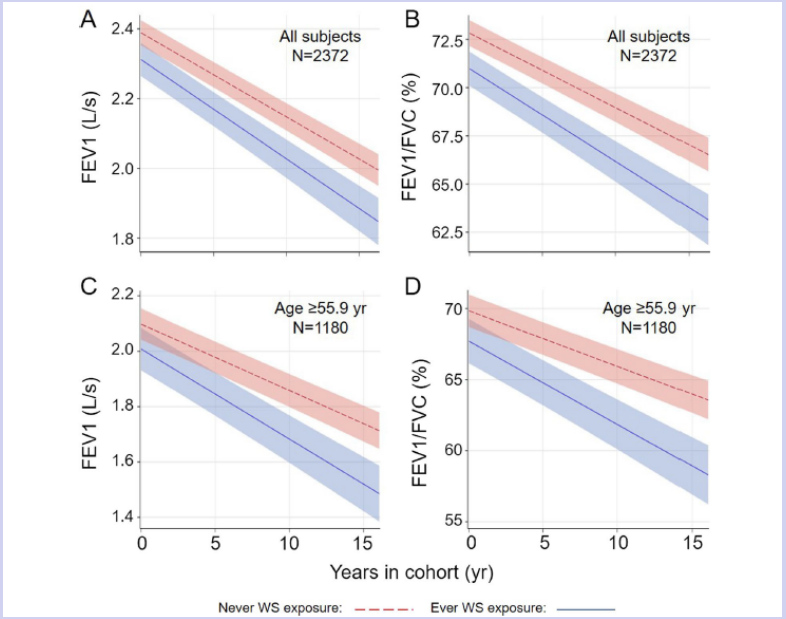
- **Definition:** Have you ever been exposed to wood smoke for a year and longer (yes or no)
- **Major findings**
  - Wood smoke exposure accelerates decline of FEV1 and FEV1/FVC ratio, but not FVC.
  - Wood smoke exposure has multi-dimensional impact on health.
  - Wood smoke exposure increases all-cause mortality partially through its adverse effects on lung health.





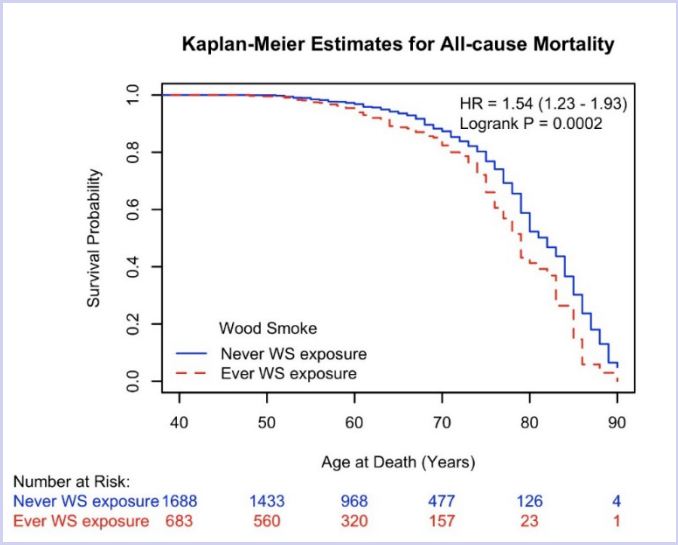


Table 4 Impact of ever WS exposure on SGRQ and SF-36 scores independent of current smoking, comorbidity, airway obstruction, and CMH status at baseline

Score	Basic model[a]		Alternative model[b]	
	Estimate (SE)	P	Estimate (SE)	P
SGRQ				
Symptom	8.5 (0.9)	< 0.0001	5.7 (0.8)	< 0.0001
Activity	8.1 (1.0)	< 0.0001	5.4 (0.9)	< 0.0001
Impact	5.0 (0.6)	< 0.0001	3.3 (0.5)	< 0.0001
Total	6.9 (0.7)	< 0.0001	4.6 (0.6)	< 0.0001
SF-36				
Physical functioning	− 7.0 (1.1)	< 0.0001	− 4.6 (1.0)	< 0.0001
Role physical	− 11.0 (1.6)	< 0.0001	− 8.1 (1.6)	< 0.0001
Bodily pain	− 6.9 (1.1)	< 0.0001	− 5.6 (1.1)	< 0.0001
Role emotional	− 6.2 (1.8)	0.0005	− 4.0 (1.8)	0.023
Social functioning	− 5.6 (1.1)	< 0.0001	− 3.9 (1.1)	0.0004
Mental health	− 3.8 (0.9)	< 0.0001	− 2.9 (0.9)	0.0009
Vitality	− 5.9 (1.0)	< 0.0001	− 4.1 (1.0)	< 0.0001
General health perceptions	− 6.1 (0.9)	< 0.0001	− 3.8 (0.9)	< 0.0001

SF-36 the short form 36 health survey questionnaire, *SGRQ* St. George's Respiratory questionnaire, *WS* woodsmoke

[a] Basic model assessed the impact of ever WS exposure on SGRQ scores using linear mixed effects model or on SF-36 scores using generalized linear model

[b] Alternative model added Charlson comorbidity score (≥ 1 versus 0), airway obstruction, and CMH at baseline into the basic model to assess the independent components of effects for ever WS exposure

Minimal difference of clinical importance = 4 for SGRQ scores

Thank you for your attention!

No covariates affecting MaCL endpoints consistently

Table 3. Associations between demographics and ever wood smoke exposure and MaCL endpoints

Variable	Comparison	NOP		AP		%CWP	
		Association	P	Association	P	Association	P
Age	Per 5 yr	0.95 (0.83 - 1.07)	0.39	0.93 (0.82 - 1.05)	0.25	-0.91 ± 1.15	0.42
Sex	Female vs male	1.01 (0.51 - 2.00)	0.98	1.00 (0.50 - 2.00)	0.99	-0.45 ± 6.50	0.95
Quit-time	<10 yr vs current	0.65 (0.41 - 1.03)	0.068	0.67 (0.43 - 4.04)	0.076	-5.76 ± 4.09	0.16
	>10 yr vs current	0.46 (0.19 - 1.12)	0.087	0.62 (0.33 - 1.15)	0.13	-8.18 ± 5.87	0.17
PY	Per 10 packyears	0.97 (0.90 - 1.05)	0.47	0.99 (0.93 - 1.06)	0.79	-0.34 ± 0.68	0.62
BMI	>25 vs ≤25	0.69 (0.48 - 1.01)	0.055	0.71 (0.48 - 1.06)	0.093	-1.33 ± 3.71	0.72
Ethnicity	NHW vs others	1.20 (0.71 - 2.03)	0.49	1.24 (0.77 - 2.00)	0.37	1.28 ± 4.65	0.78
Woodsmoke exposure	Ever vs never	1.23 (0.82 - 1.84)	0.32	1.27 (0.85 - 1.91)	0.25	8.73 ± 3.91	0.028