SARS-CoV-2 Biosensor Development: Exhaled Particle Measurements Using Optical Particle Counter, Quadrupole Mass Spectrometer, and Quartz Crystal Microbalance

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Breath Background

Detecting respiratory diseases from the breath is a field with high potential and is underdeveloped

Divisions of Breath Currently Analyzed

1. Gaseous breath
2. Volatile Organic Compounds (VOCs)
3. Exhaled Breath Condensate (EBC)
   - Aerosolized droplets of respiratory-tract lining fluid (RTLF) containing trapped particles
   - Water vapour consisting of breath humidity

Our novel approach only considers exhaled RTLF microdroplets on a biosensor without collecting EBC

Respiratory Tract Lining Fluid (RTLF) Microdroplets

- Deep expirations cause small bronchiole airways (<2mm) to collapse and seal with RTLF. Inspiration distends these small airways which generates aerosolized RTLF microdroplets [1]
- Laryngeal activity such as talking, coughing, sneezing, and high-velocity gas flow have been demonstrated to produce RTLF microdroplets [1, 2]
- **Aerosol RTLF microdroplets are expelled out of the body and can be collected for respiratory system analysis** [1]

SARS-CoV-2 in RTLF

- Growing amounts of evidence is supporting aerosol transmission of COVID-19 because **SARS-CoV-2 is active in airborne microdroplets** (<5um) [2,3]
- Not only does coughing, sneezing, and dyspnea generate aerosol microdroplets, but evidence is supporting that **regular breathing generates microdroplets** as well [2,3]
- Capturing these microdroplets with a biosensor has the potential for a novel breath-based COVID-19 test, as well as other respiratory viruses

Project Overview

Develop a SARS-CoV-2 biosensor to analyze exhaled microdroplets

Short-Term Research Goals

1. Investigate the nature of exhaled particles
   - with respect to air quality, relative humidity, temperature
   - using an optical particle counter (OPC), quadrupole mass spectrometer (QMS), and a quartz crystal microbalance (QCM)
2. Establish a process to collect and analyze breath samples for their microdroplets
3. Successful direct deposition of exhaled microdroplets on a hydrophilic crystal in a vacuum environment for surface-science analysis

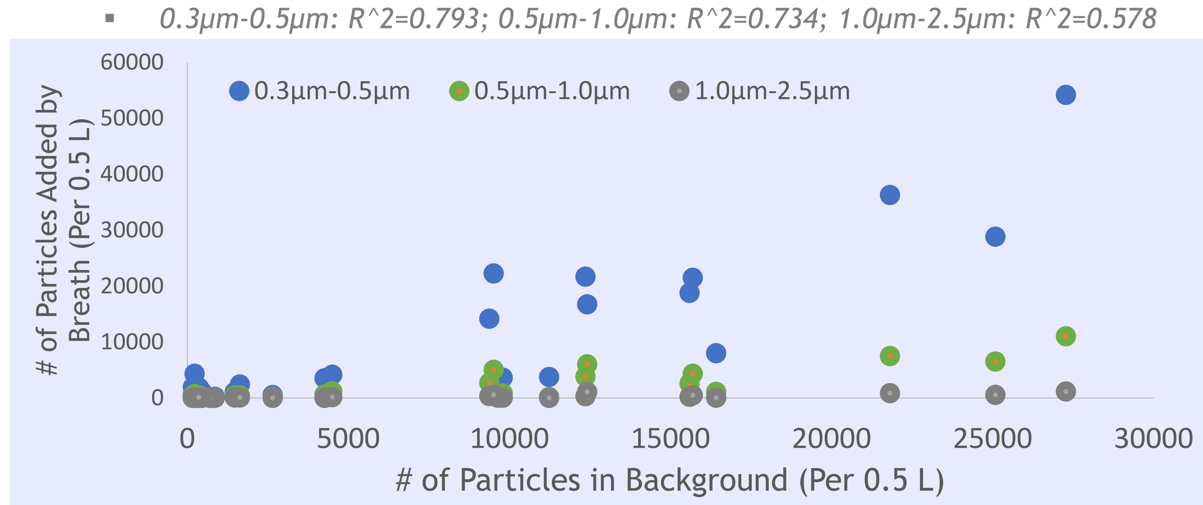
Optical Particle Counter Research

Methodology

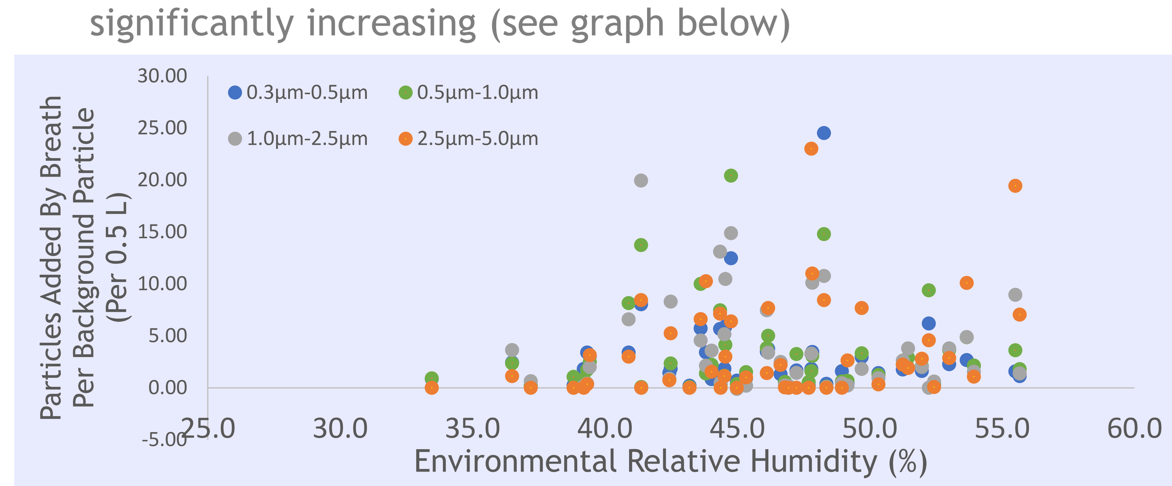
- Background particle concentrations were measured
- Sample bag collects exhale via disposable mouthpiece
- Deep expirations used to inflate sample bags
- PCE-PQC 10 attaches to valve to measure aerosol microdroplets
- Six channels of particle sizes are recorded (values only refer to interval and are not cumulative)
- Minimum particle size measurable is 0.3µm and max is 10.0µm
- Multiple 0.5L breath samples taken per bag
- All tubing, valves rinsed with high-pressure Nitrogen

Results

- In 30 Trials considering 0.3µm-1.0µm (RH=45%-55%), there are an average of **356% particles of the background in the breath**
  - Standard deviation of 368%, maximum of 2551%, minimum of 104%
  - 6/30 Trials less than 150%, 26/30 trials less than 500%
- **Particles in the background are positively correlated with the number of particles added in the breath** (see graph below)
  - *0.3µm-0.5µm: R^2=0.793; 0.5µm-1.0µm: R^2=0.734; 1.0µm-2.5µm: R^2=0.578*

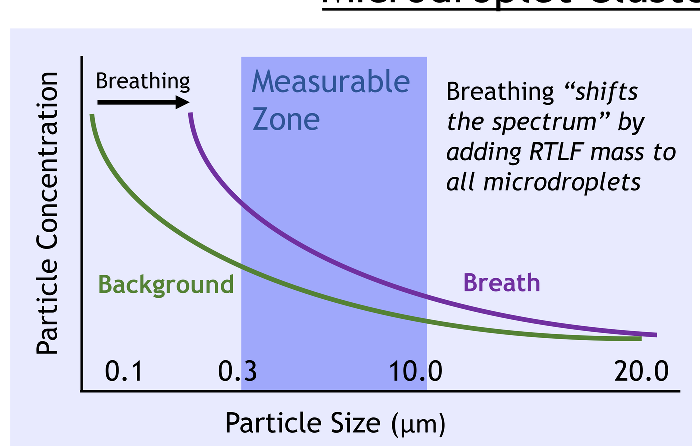


- **Low relative humidity (RH) consistently had a smaller increase in particle counts in breath.** High RH was more varied with some significantly increasing (see graph below)



Conclusions:
- More microdroplets are in the breath than in the background air
- More particles are added in dirtier air than in clean air
- Low relative humidity reduces the number of particles exhaled

Microdroplet Clustering Theory



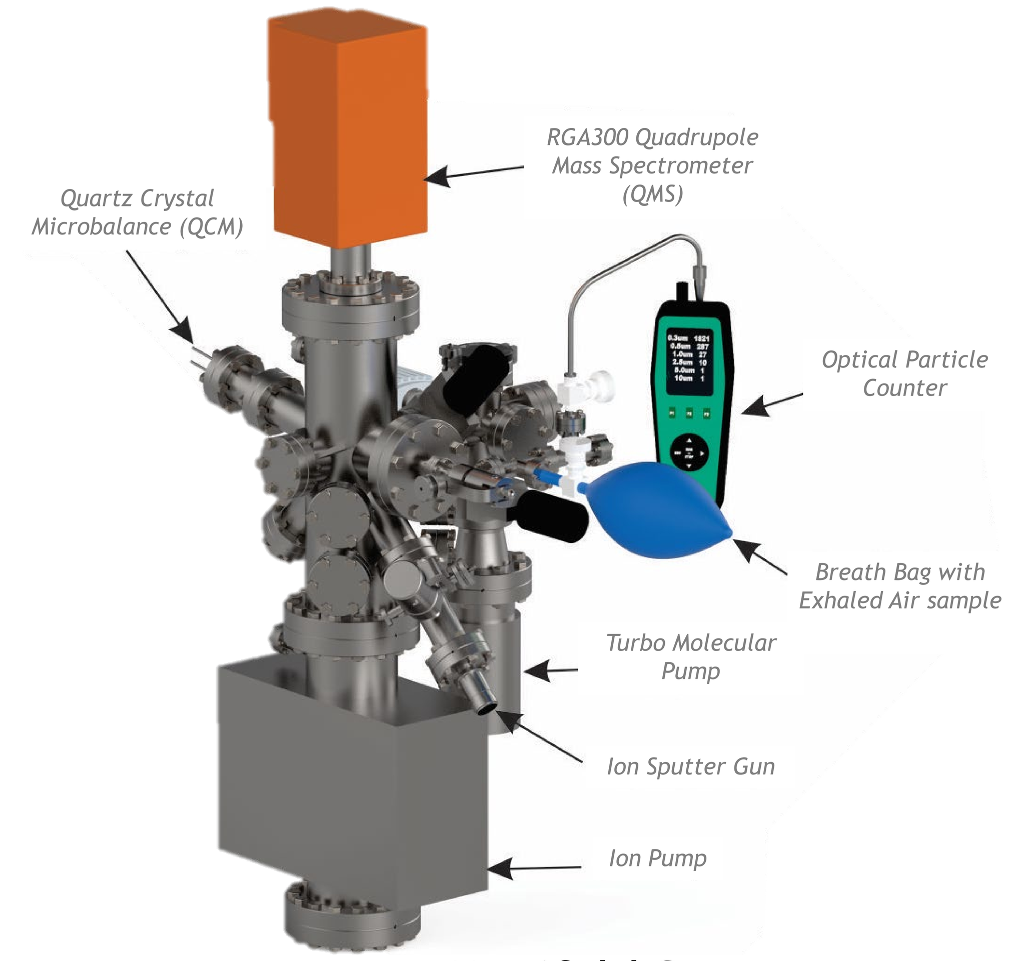
- Particle concentration rapidly increases with decreasing size
- Only one band in the spectrum is measurable due to tool limitations
- When water-based ambient microdroplets are inhaled, they **pass through airways that are "wet"**
- **Microdroplets increase in size because they pick up some RTLF**
- This shifts the particle spectrum, which makes the more prevalent <0.3µm particles visible in our measurement window, increasing counts

Supporting Microdroplet Clustering Theory with Results

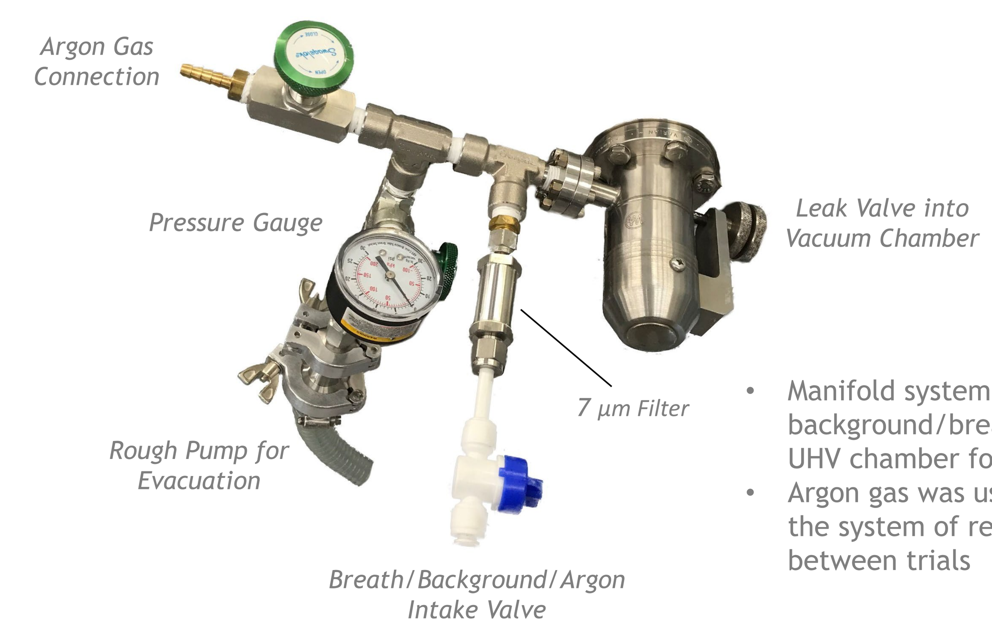
Higher Background Counts Yield a Greater Increase
- More particles in the background provides more media for RTLF to cluster with
- Exponential nature of curve explains why a greater increase is observed when larger particles are measured in the background

Relative Humidity (RH) Correlation
- Low RH suggests smaller water microdroplets in the background, RTLF spectrum shift will have a lesser effect

Ultra-High Vacuum (UHV) System Setup



Manifold System



- Manifold system admits background/breath samples into UHV chamber for QMS & QCM
- Argon gas was used to rinse out the system of residual particles between trials

The 7 µm filter is used to reduce the flow of the sample into the system. This allows for the leak valve to be opened further to achieve the same pressure. Trials typically increased the vacuum system from 1e-7 torr to 5e-6 torr.

Quadrupole Mass Spectrometer (QMS)

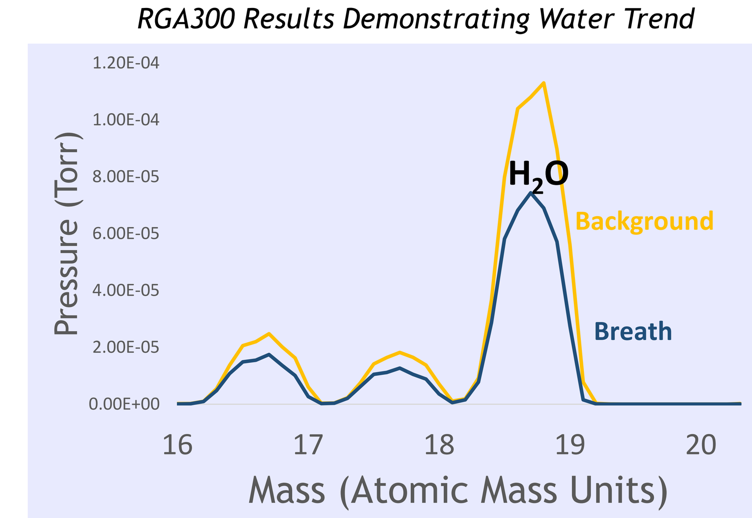
A trend appeared of less gaseous water in breath than the background

- RGA300 yielded varied results in which the **most had a decline in water concentration in the breath** (RH ~40%)
- **11/14 QMS trials had less water in the breath with an average decline of 4.19%** (Water reduction per initial pressure)
- Possible error: hydrogen concentrations in some trials varied dramatically (0.5% to 4144.4% of initial pressure)

Result Interpretation

- The lesser gaseous water in the **breath suggests that water vapour is condensed during breathing**
- **Humidity may contribute to the "spectrum shift"** in the cluster theory
- We cannot distinguish how much mass is contributed to microdroplets from humidity versus RTLF
- The presence of SARS-CoV-2 in breath confirms that RTLF has some degree of influence

RGA300 Results Demonstrating Water Trend



Quartz Crystal Microbalance (QCM)

No consistent data was collected because of challenges with microdroplets in the UHV system

- A vertical and horizontal QCM was minimally influenced by breath samples in the manifold setup due to certain identified challenges

Challenges For Collecting Microdroplets on QCM in UHV

1. The **decrease in temperature** from the breath to the room-temperature UHV induces microdroplet condensation/settling
2. **Two ninety-degree turns** in the manifold system contributes to loss of particles, particularly heavier particles
3. **Hydrophobicity of QCM surface** causes immediate evaporation of microdroplets

Challenge Resolutions for Future Testing

1. Heat manifold and bag to reduce loss to condensation
2. Replace manifold system with a straight-path system for microdroplets
3. Deposit hydrophilic film on QCM to discourage evaporation

Next Steps

- Deposit microdroplets on a crystal and establish preserved crystallographic structure
- Research receptive films (aptamer, antibody, etc.) on established crystals for SARS-CoV-2
- Develop transducer to identify SARS-CoV-2 presence

OCI Vacuum Microengineering Inc. has researched the water-resilient properties of $LiNbO_3$ making it a strong candidate. See @o_guenevere's #AVSPosters2020 for details

General Conclusions

- OPC tests confirm the possibility of significant exhale particle generation (356% of inhaled air)
- High concentration of background particles induce a greater difference in exhale to inhale than low particle concentration background
- Less gaseous water is in exhale than in inhaled air
- Low RH weakly decreases the amount of particles added by the breath
- Measuring exhaled microdroplets using a QCM is a challenging process

COVID-19 Conclusions

- Our OPC trials demonstrate that **we exhale significantly more particles than we inhale**
- Our results, combined with current research [2,3] create a compelling case that **SARS-CoV-2 can spread via aerosol transmission during normal breathing**
- This evidence has a serious impact on the transmission of any infectious airborne pathogens and relevant suppressing protocols
- Further research can develop a SARS-CoV-2 biosensor that could **analyze breath for COVID-19**

In future conclusions, the lungs of infectious people may multiply the viral microdroplet concentration in the air

References

[1] B. Bake, P. Larsson, G. Ljungkvist, E. Ljungström, and A.-C. Olin, "Exhaled particles and small airways," *Respiratory Research*, vol. 20, no. 1, 2019.
[2] N. M. Wilson, A. Norton, F. P. Young, and D. W. Collins, "Airborne transmission of severe acute respiratory syndrome coronavirus-2 to healthcare workers: a narrative review," *Anaesthesia*, 2020.
[3] M. Yao, L. Zhang, J. Ma, and L. Zhou, "On airborne transmission and control of SARS-Cov-2," *Science of The Total Environment*, vol. 731, p. 139178, 2020.