DEVELOPMENT OF REAL TIME REACTIVE OXYGEN SPECIES MONITORS

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Queensland University of Technology

International Laboratory for Air Quality and Health
WHO Collaborating Centre

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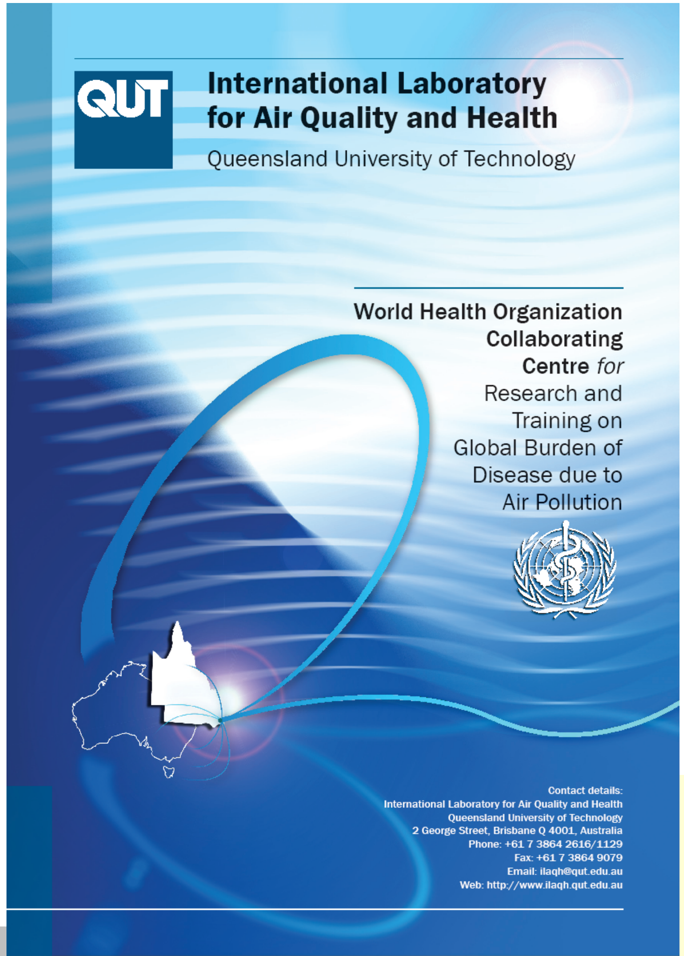
Lidia Morawska



....since 1993

....until 2003, Environmental Aerosol Laboratory

....part of IHBI

....since 2004, WHO Collaborating Centre

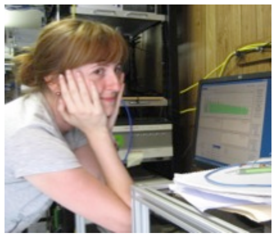
....multidsciplinary team of over 25 researchers

Free Radical Chemistry and Biotechnology

Steven Bottle

An Australian Research Council Centre of Excellence

www.freeradical.org.au



Branka Miljevic

5 Universities, >100 Scientists, >$28 M budget

Specialist MS, Laser, Synthesis, Magnetic Resonance and Fluorescence Equipment

Svetlana Stevanovic

FREE RADICAL CHEMISTRY BIOTECHNOLOGY an ARC Centre of Excellence

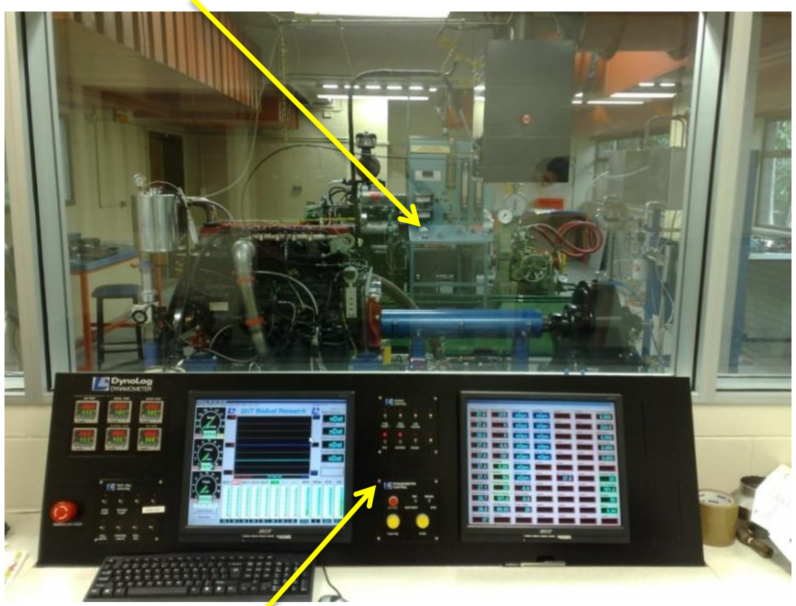
QUT labs: Headed by Prof. Steven Bottle, with Prof. Zoran Ristovski (Associate) and Drs. Miljevic and Fairfull-Smith, 1 PD and 12 PhD students

Australian Government Australian Research Council

Biofules Engine Research Facility - BERF

Richard Brown

Engine (s)



CONTROL ROOM

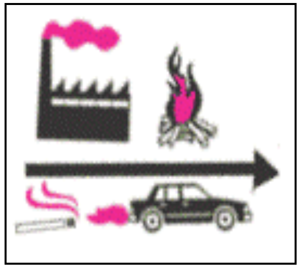
CAPABILITIES

- Fuel Handling
  - Liquid and gaseous fuels (Hydrogen)
  - Dual fuel capability
  - Separate Biofuel / Petroleum handling
- Emission Tests
  - Gas (CO_2, CO, HC, NO_x)
  - Particles

PM & health effects

- Epidemiological studies - strong associations between levels of ambient particulate matter (PM) and increased respiratory and cardiovascular disease morbidity and mortality
- mechanism(s) by which particles induce adverse health effects are still not entirely understood

Proposed mechanism: Oxidative stress hypothesis



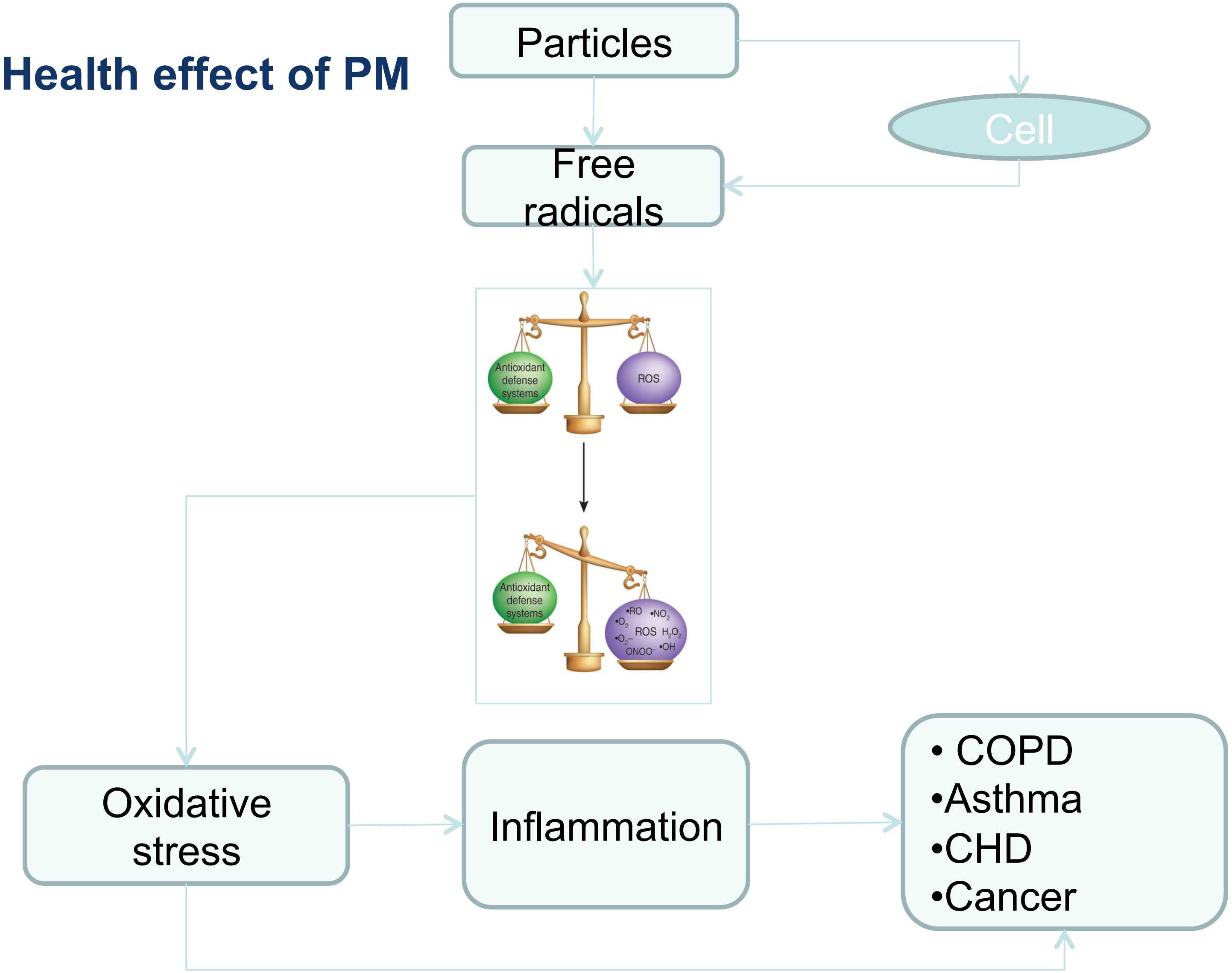
PM → free radicals; ROS → oxidative stress → inflammation

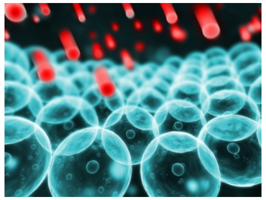
↓

cell injury / death

Ristovski, Z., Miljevic, B., et al., (2012). Respiratory health effects of diesel particulate matter. *Respirology*, *17*, 201–212.

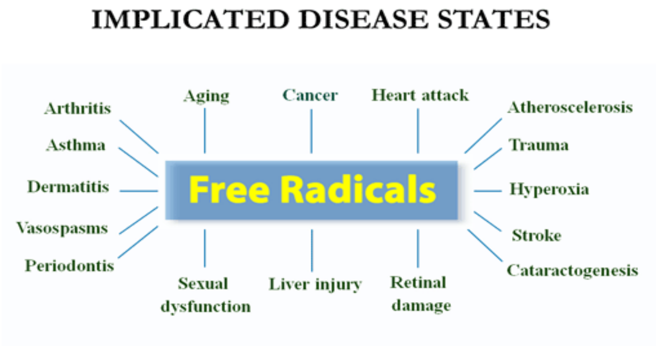
Health effect of PM





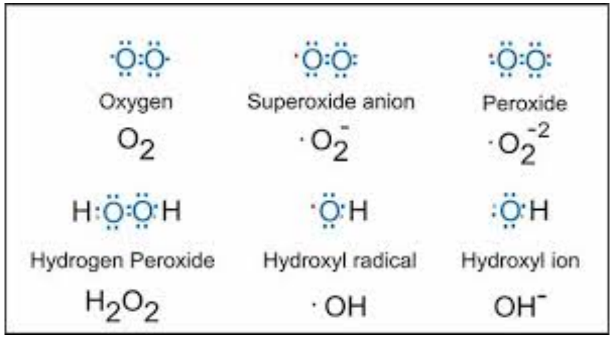
Free radicals

- Free radicals can be generated in the human body
- Free radicals make chain reaction
- Antioxidant terminate the chain reaction



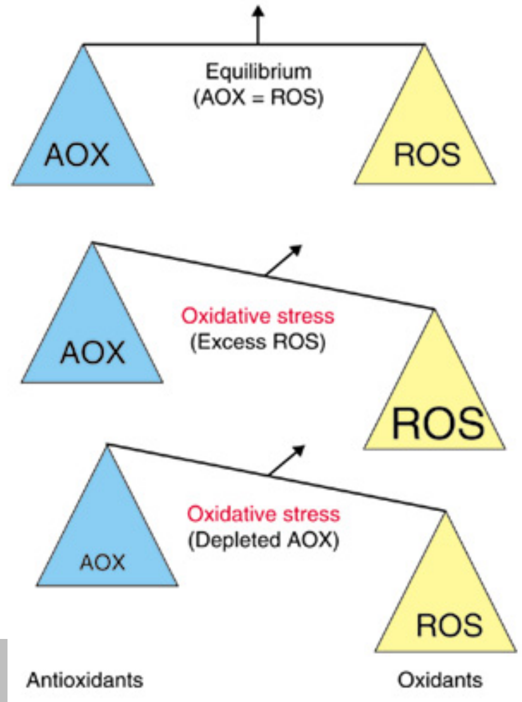
ROS

- Sources: Exogenous and Endogenous
- Oxidative stress



PM & health effects

- **ROS** – Reactive Oxygen Species ($O_2^{-\cdot}$, $HO\cdot$, $RO\cdot$, $ROO\cdot$, $^1O_2$, $ONOO^-$, H_2O_2, ROOH)
- **oxidative stress -** an imbalance between the production of ROS and the cell's (or body's) natural antioxidant defence.



Endogenous and exogenous ROS

- ROS can be formed **endogenously**, by the lung tissue cells, during the phagocytic processes initiated by the presence of PM in the lungs, or by particle-related chemical species that have the potential to generate ROS.
- In addition to the particle-induced generation of ROS, several recent studies have shown that particles may also contain ROS (so called, **exogenous** ROS).

Measurements of the radical generation capacity of the PM

Toxicological studies

- **In vivo**
- **In vitro**
- **Acellular assay**

In vivo

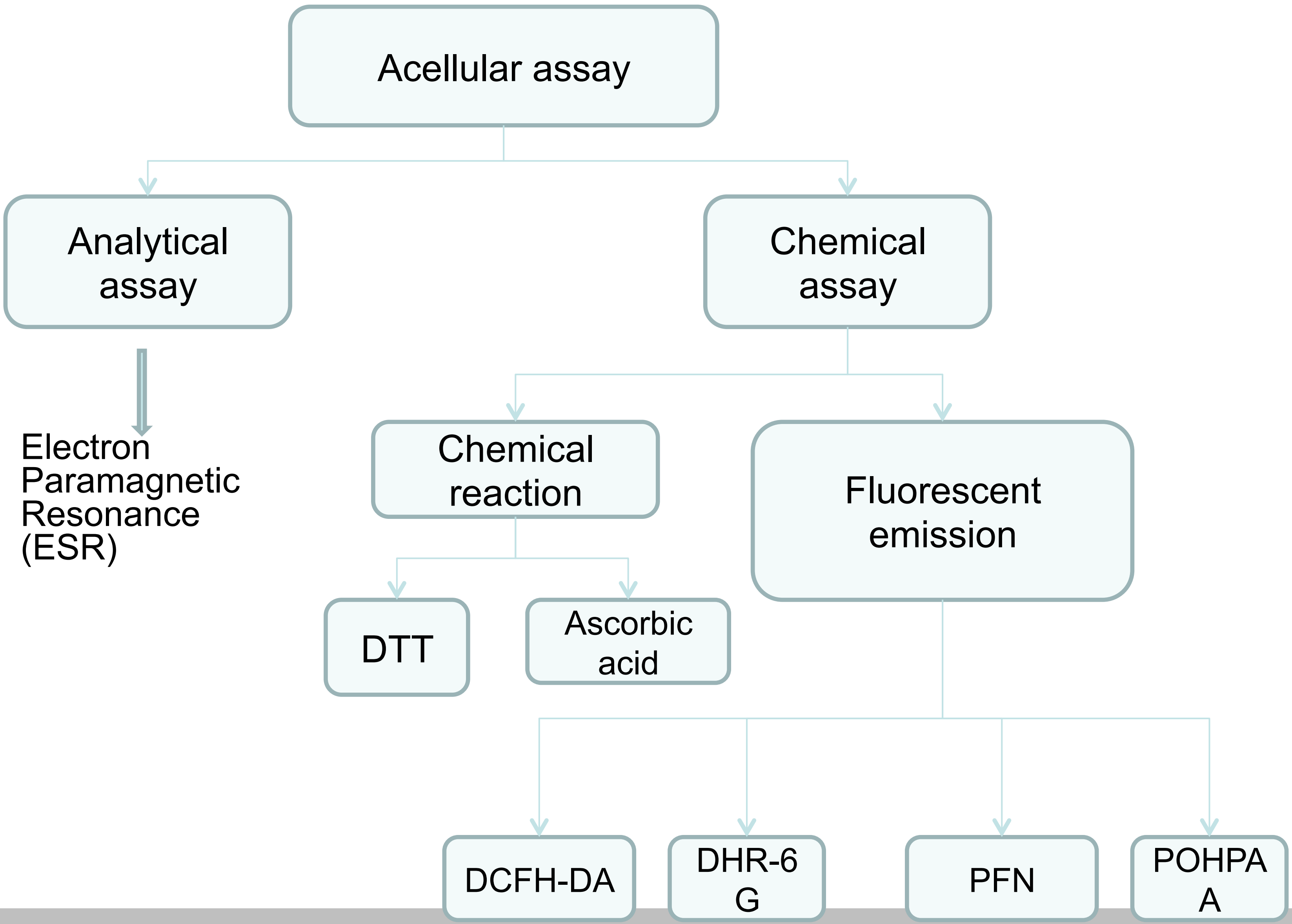
- ***The best predictor of PM toxicity***
- *Complicated*
- *Expensive*
- *Time-consuming*
- *Ethical approval*
- *Not suitable for routine analyses and field studies*

In vitro

- ***Alternative for in vivo studies***
- *Complicated*
- *Expensive*
- *Time-consuming*
- *Not suitable for routine analyses and field studies*

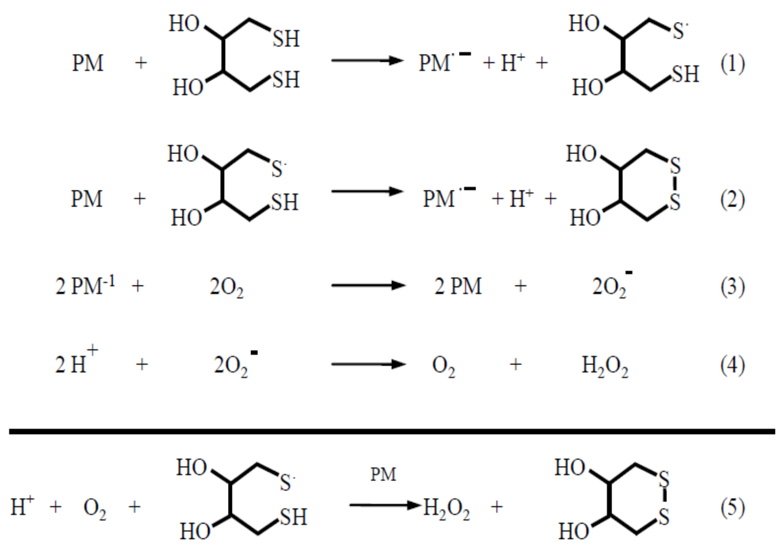
Acellular assay

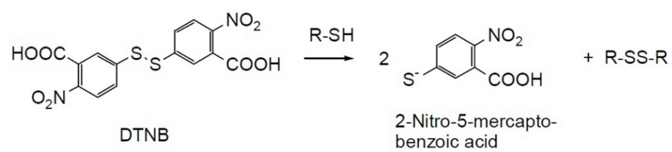
- ***Cheaper; Faster***
- ***No need for ethical approval***
- ***Easily applied in field studies and routine analyses***
- *Only inherent oxidative potential; No biological interaction*


Acellular assay
Analytical assay
Chemical assay
Electron Paramagnetic Resonance (ESR)
Chemical reaction
Fluorescent emission
DTT
Ascorbic acid
DCFH-DA
DHR-6 G
PFN
POHPA A

DTT

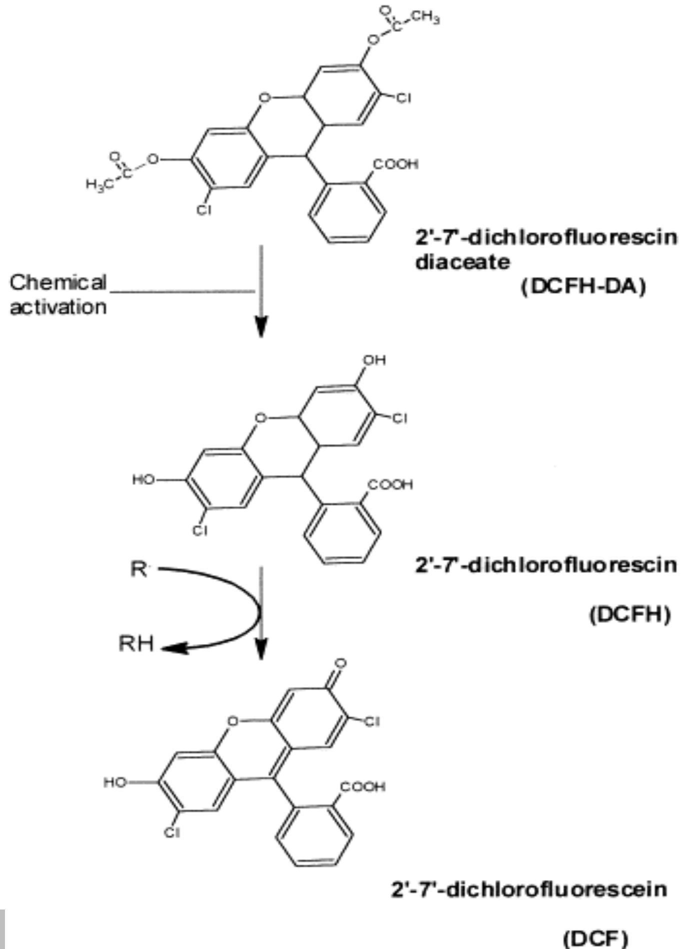
- Dithiotheritol
- Strong reducing agent
- Measure the formation of the ROS by quinones
- Remaining DTT reacts with Ellman reagent
- Following by TNB production in 412 nm
- Report as:
  - nmol DTT $min^{-1} \mu g_{PM}^{-1}$
  - normalized index of oxidant generation and toxicity (NIOG)
- *DTT and TNB are both light sensitive*
- *DTT is reactive toward limited number of species*
- *Needs incubation time up to 90 min*





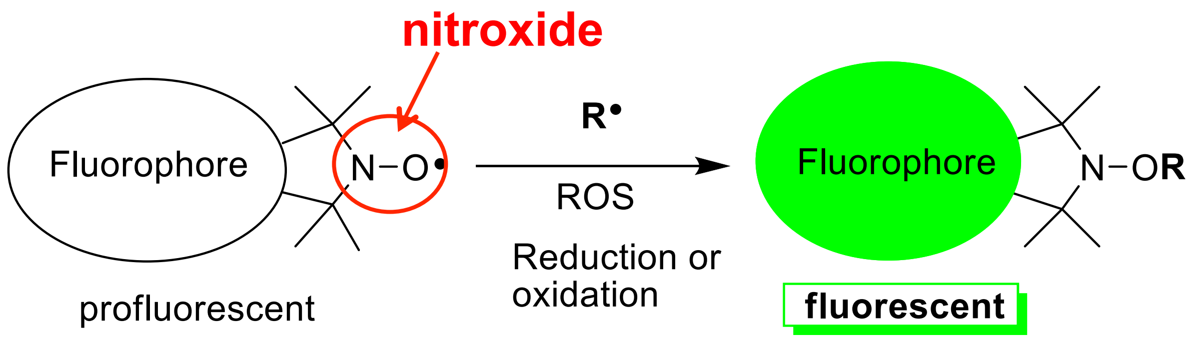
DCFH-DA

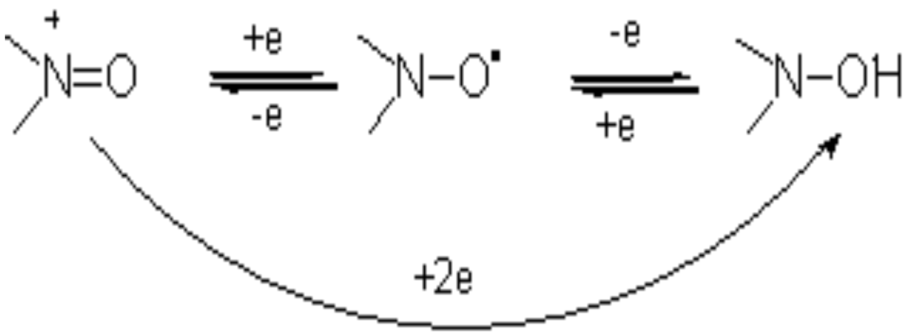
- Dichlorofluorescin Diacetate
- Emit fluorescence after being oxidized by hydrogen peroxide
- Detection of H_2O_2
- Needs catalyst usually HRP
- Calibration curve base on hydrogen peroxide equivalent particles
- *Prone to outooxidation*
- *HRP make three fold increase in the fluorescent intensity*



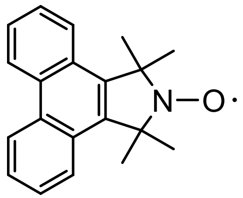
Profluorescent nitroxides

- Supressed fluorescence emission in the presence of nitroxide moiety
- These molecules react with radicals, leading either to reduction of the nitroxides to the hydroxylamines or oxidation to oxoammonium cation



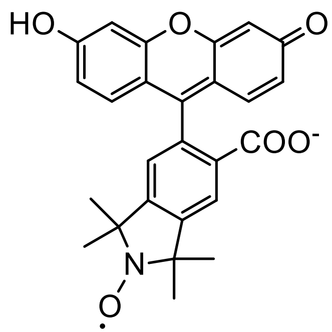


Profluorescent nitroxides synthesised at QUT



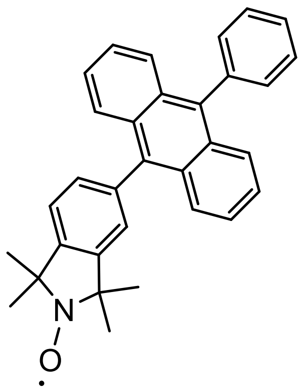
Phenanthrene-nitroxide

λ_{ex}= 294 nm
λ_{em}=355 nm
372 nm



Flourescein-nitroxide

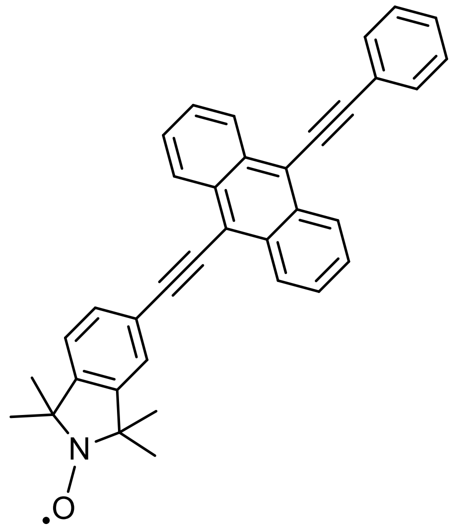
λ_{ex}= 495 nm
λ_{em}=515 nm



9,10–diphenylanthracene-nitroxide

λ_{ex}= 395 nm
λ_{em}=410 nm
430 nm

BPEAnit



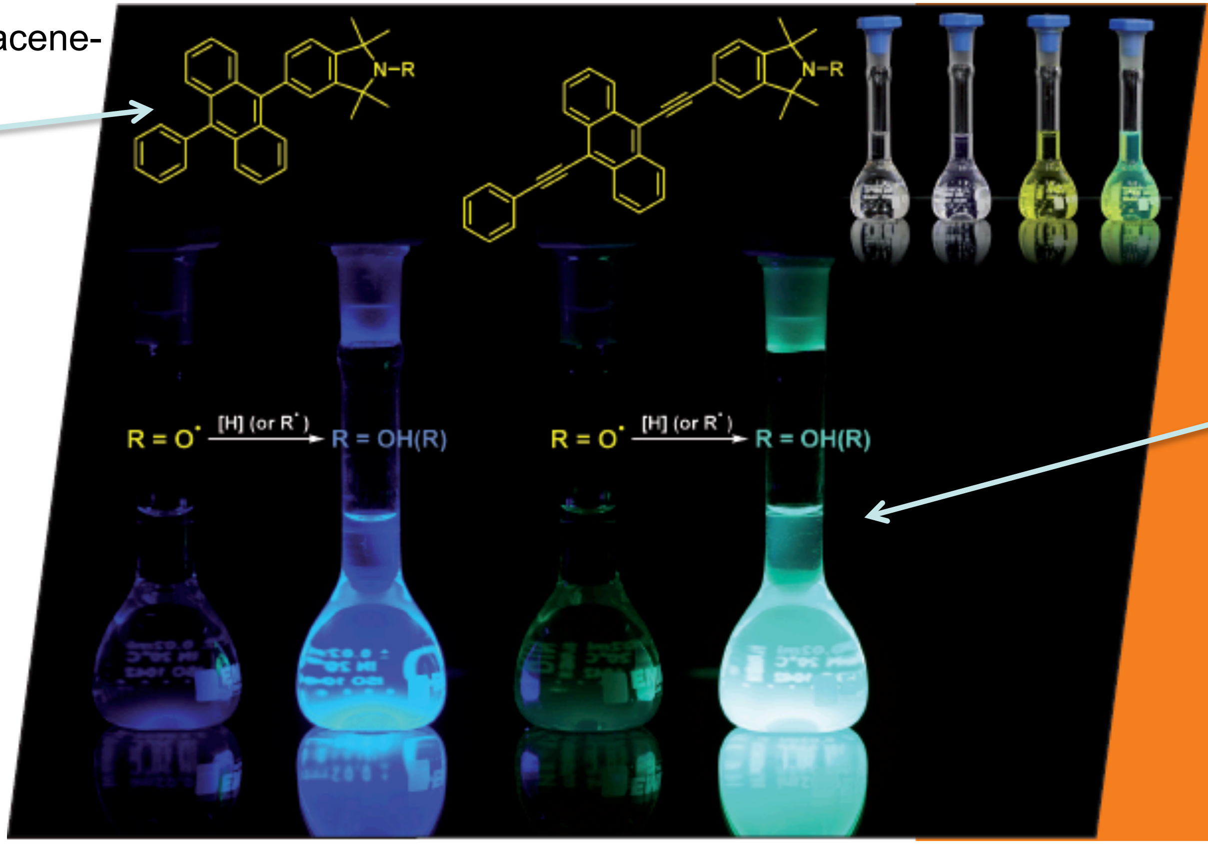
9,10-bis(phenylethynyl)anthracene-Nitroxide (BPEAnit)

λ_{ex}= 430 nm
λ_{em}=485 nm
510 nm

Fairfull-Smith and Bottle. Eur J Org Chem (2008) (32) pp. 5391-5400

Profluorescent nitroxides (PFNs)

9,10–diphenylanthracene-Nitroxide
λ_{ex}= 395 nm
λ_{em}=410 nm
430 nm



9,10-bis(phenylethynyl)anthracene-Nitroxide (BPEAnit)

λ_{ex}= 430 nm
λ_{em}=485 nm
510 nm

Fairfull-Smith and Bottle. Eur J Org Chem (2008) (32) pp. 5391-5400

MOTIVATION

Develop a cell-free assay for rapid and routine screenings of the oxidative potential of PM.

Develop a stationary continuous monitor.

Implement the assay in a portable personal exposure sensor.

Particle sampling

Offline Sampling

Filter

- ***Low cost and higher sample volume***
- ***Excellent collection efficiency***
- *Difficult PM extraction*
- *Evaporation of the semi VOCs*
- *Ageing of the particles*
- *Need sonication to extract the particles*

Liquid impingement

- ***Rapid and direct reaction with quenchers***
- ***Low chemical changes***
- ***No ageing***
- *Not for automated use*

BPEAnit assay – sampling:

- bubbling aerosol through an impinger with fritted nozzle tip containing BPEAnit solution (DCFH) fluorescence measurement
- solvent – dimethylsulfoxide (DMSO)
- test & HEPA-filtered control sample taken

- I_{485nm}(test) - I_{485nm}(ctrl) → $I_{485nm}(ROS_{particle})$

↓

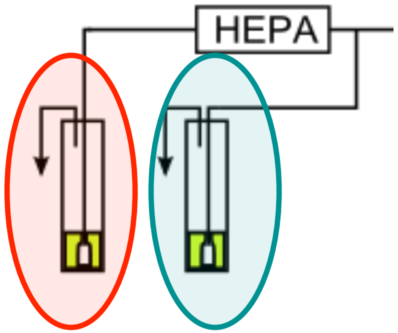
calibration curve

↓

$n\ (ROS_{particle})$

↓

Normalized to the measured particle mass

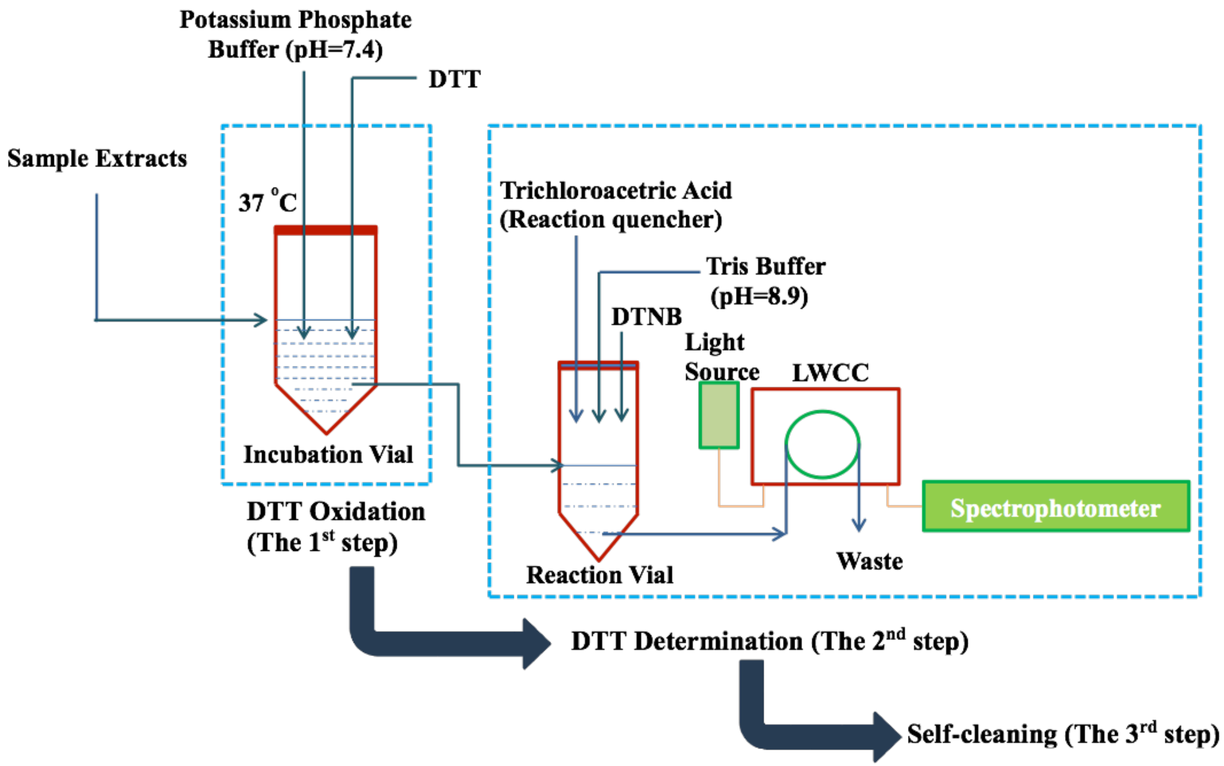


Real Time Detection of ROS

- Semi-automated systems where particles are collected onto filters first (DTT)
- To skip the extraction procedures particles have to be collected directly into a liquid
  - Water – DCFH
  - DMSO – BPEAnit
- The chemical assay can either be in the collection liquid or can be added later to the collection liquid.

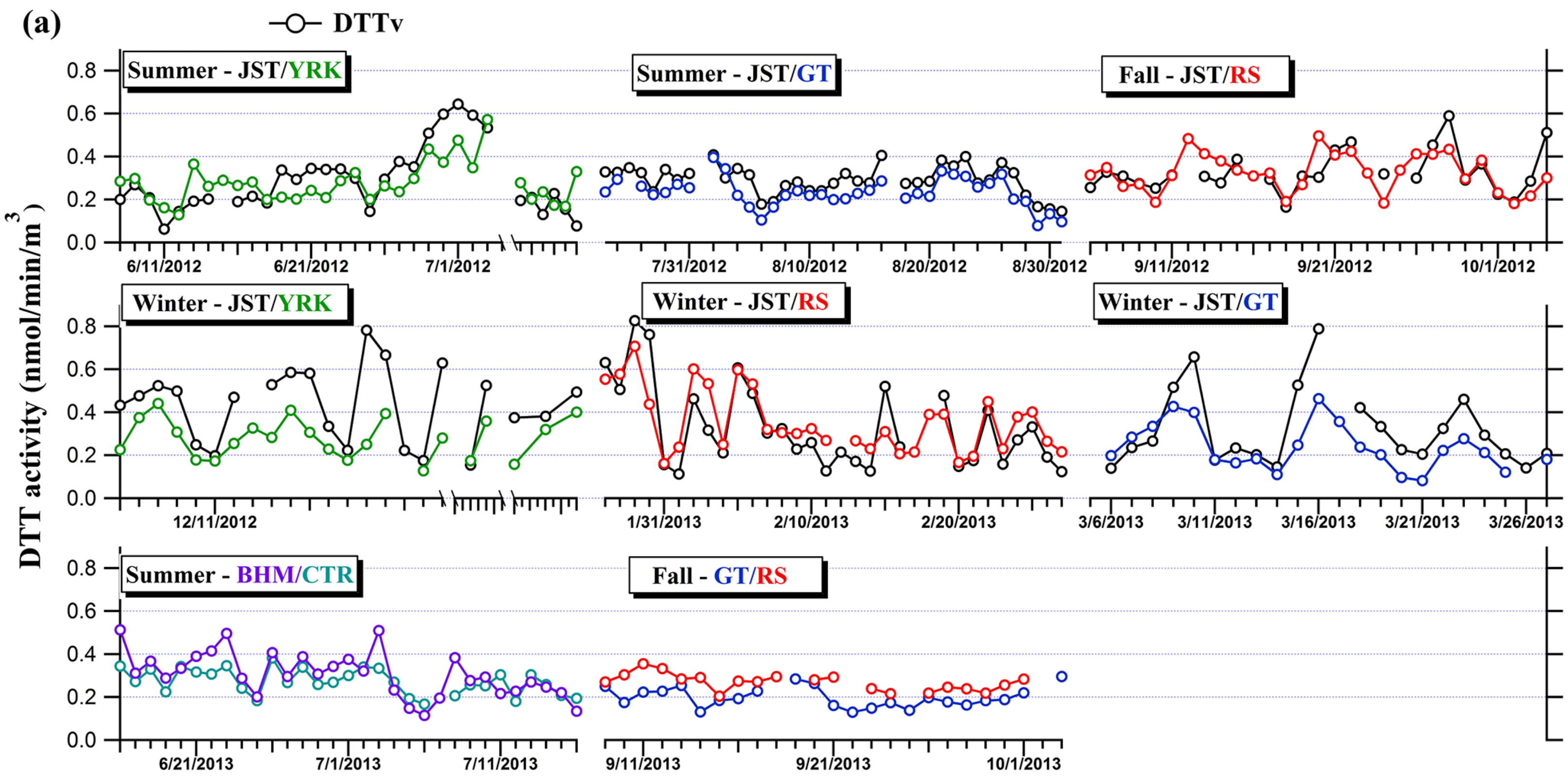
Semi-automated systems

Particle first collected on quartz filters over 23 h. Extraction procedure: 1-inch-diameter punches were extracted in 15 mL of DI water by **sonication**.
5 mL were filtered and used in the DTT assay.

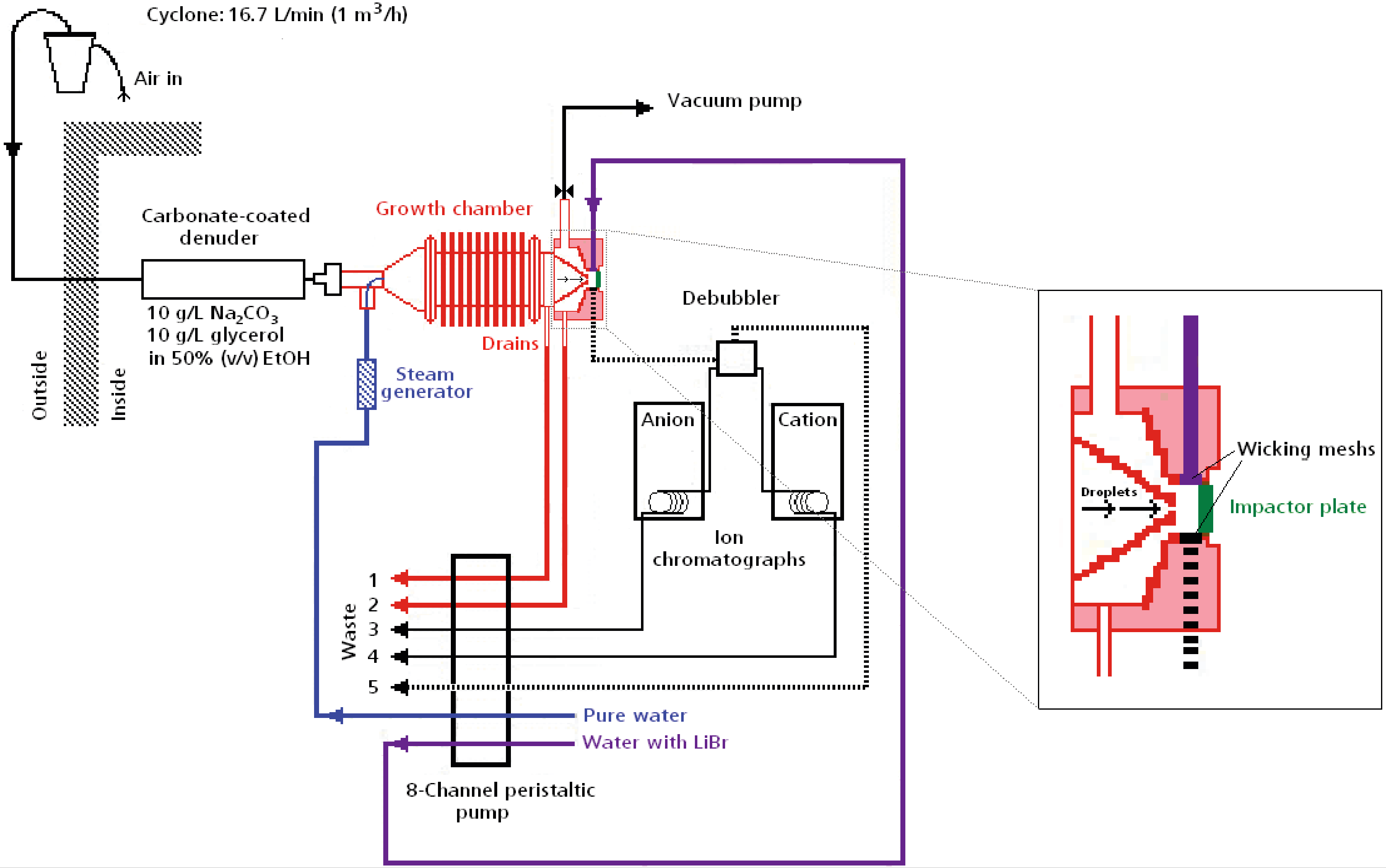


Fang, T.et al. (2015). *Atmospheric Measurement Techniques*, *8*(1), 471–482.

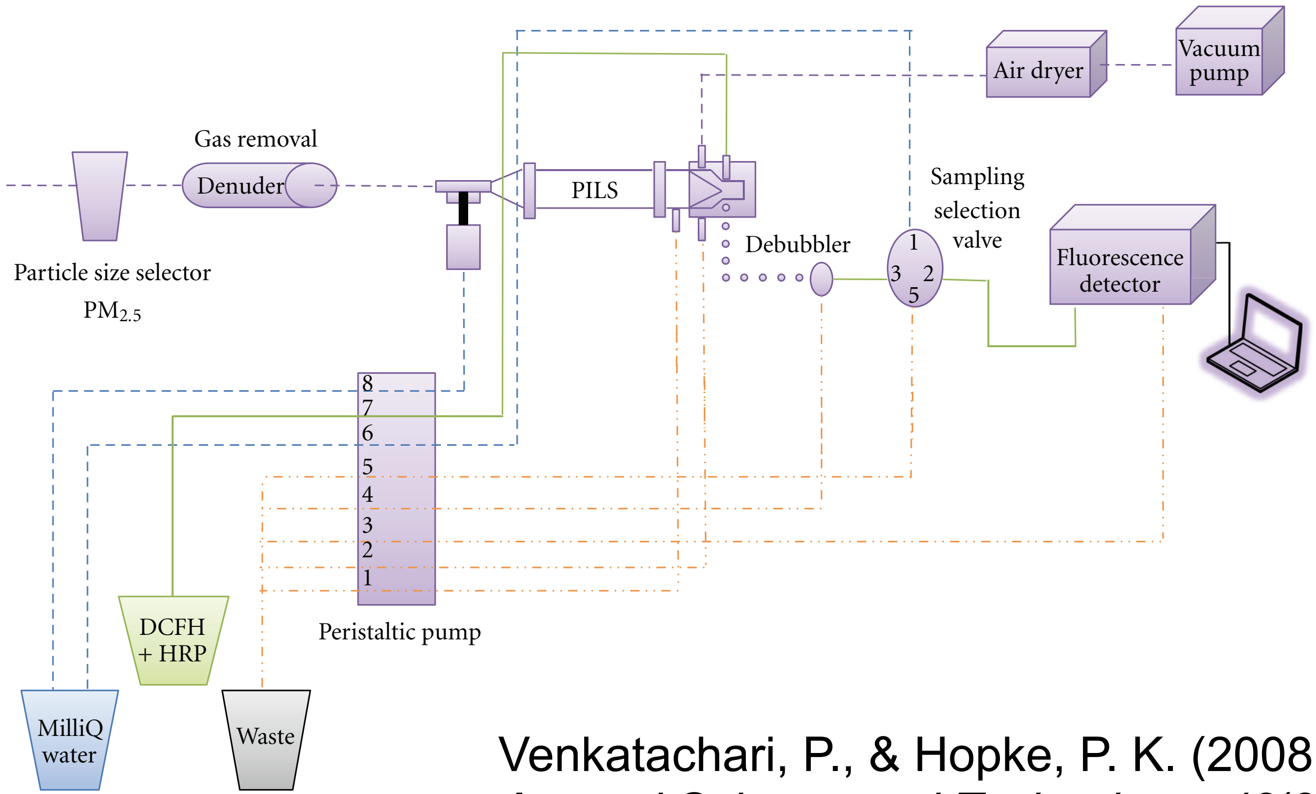
Semi-automated systems



Automated systems based on PILS

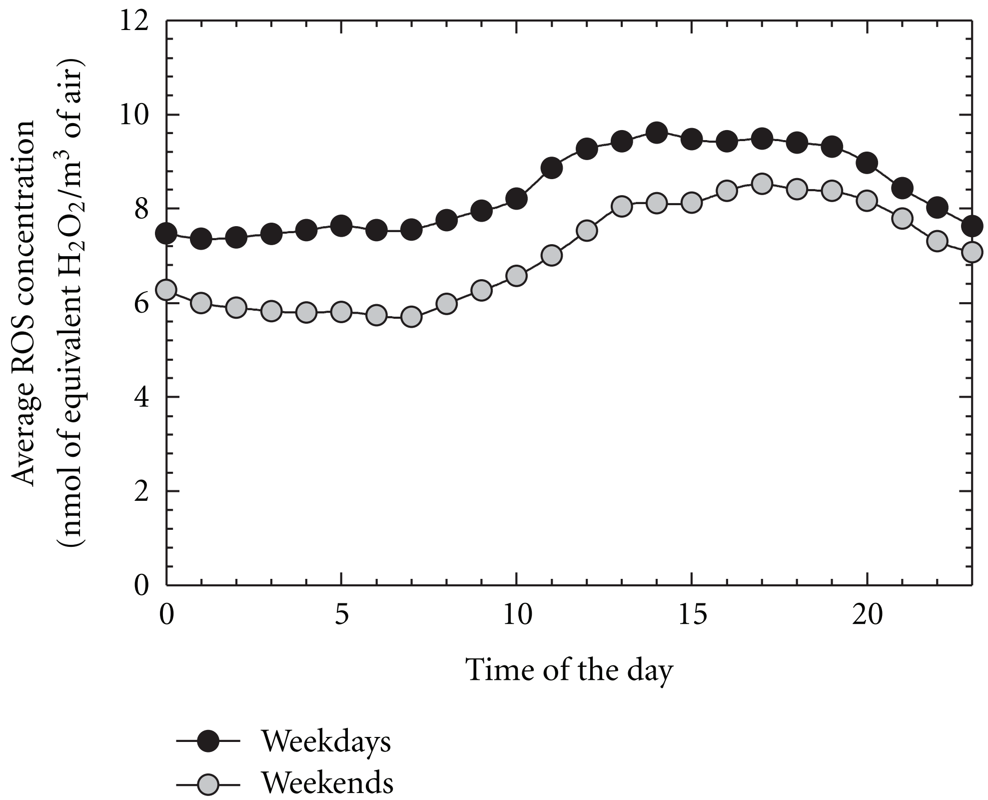


DCHF based systems – Clarkson Uni



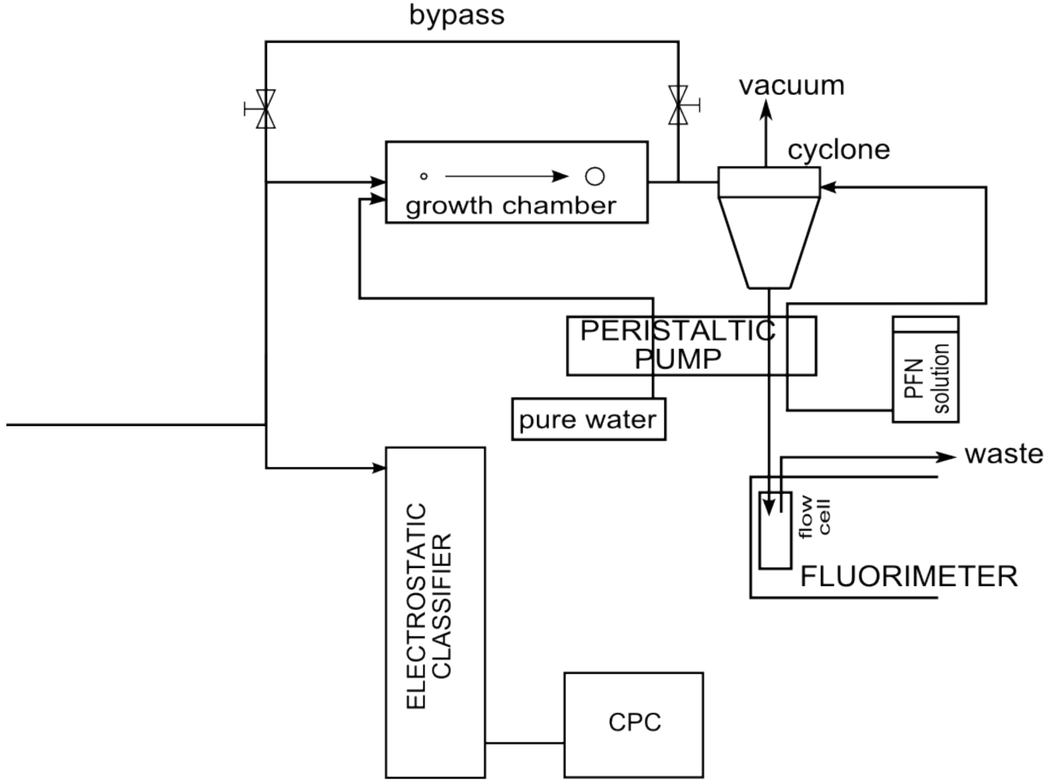
Venkatachari, P., & Hopke, P. K. (2008).. *Aerosol Science and Technology*, *42*(8), 629–635

Field results



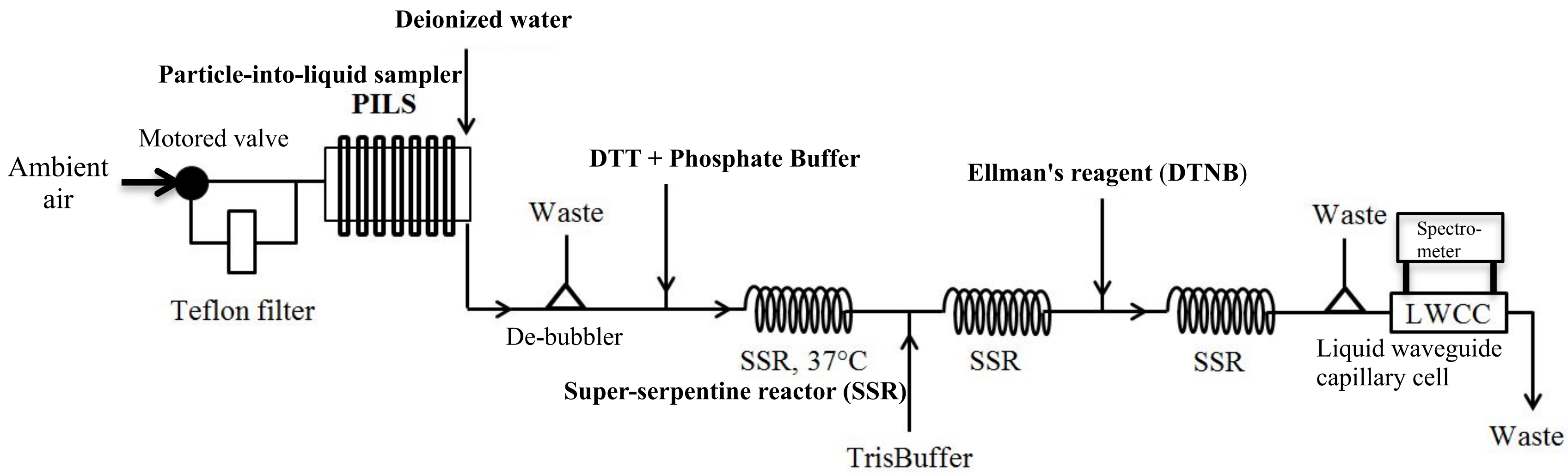
Wang, Y., Hopke, et al. (2011). Laboratory and Field Testing of an Automated Atmospheric Particle-Bound Reactive Oxygen Species Sampling-Analysis System. *Journal of Toxicology*, *2011*(ID 419476)

PFN based system in development - QUT

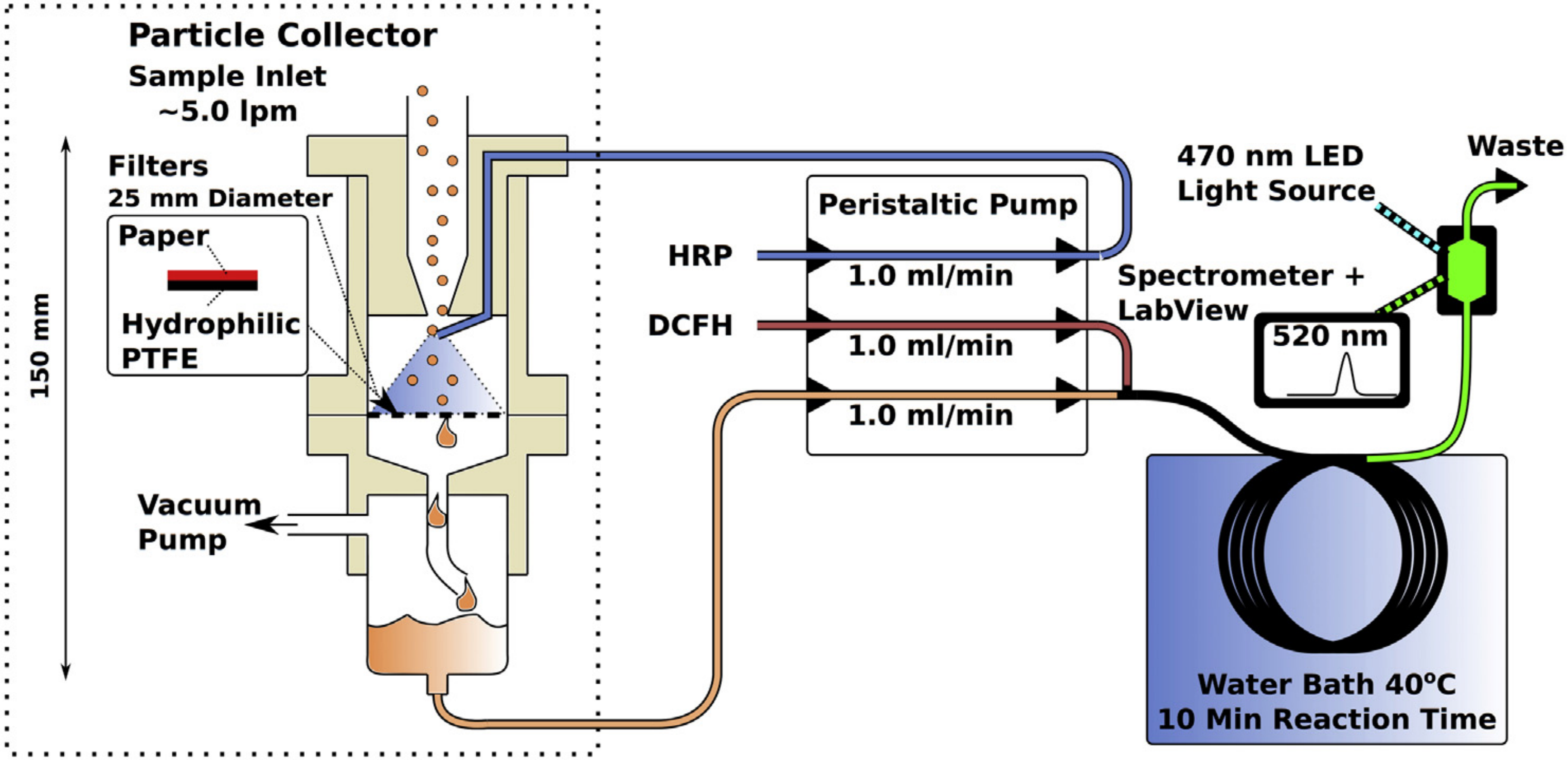


Orsini, D. A. et al. (2008). A water cyclone to preserve insoluble aerosols in liquid flow - An interface to flow cytometry to detect airborne nucleic acid. *Aerosol Science and Technology*, *42*(5), 343–356.

DTT based systems in development - University of Illinois Urbana Champaign

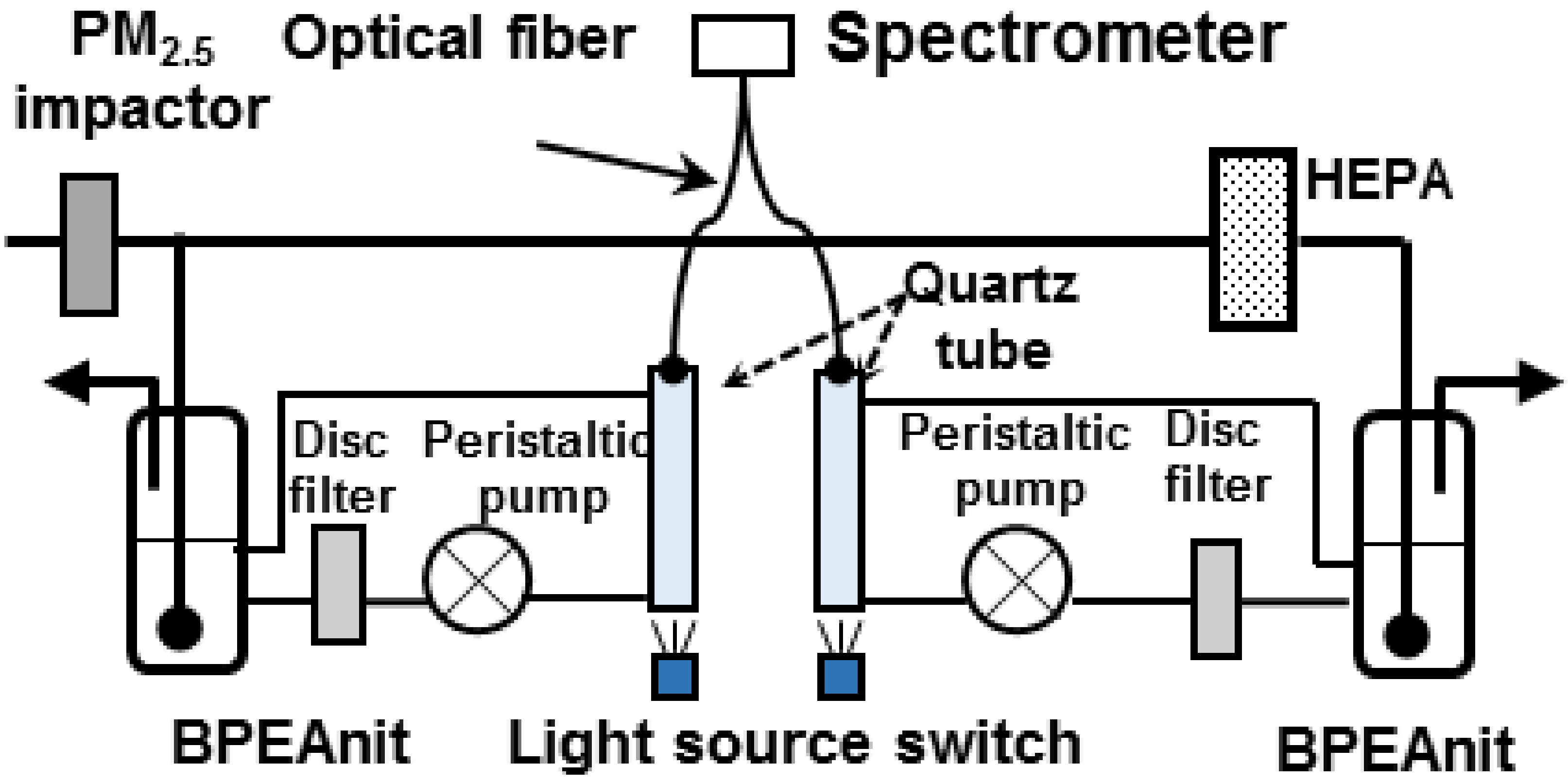


Other non PILS based systems – DCFH (Cambridge Uni)



Fuller, S. J., et al (2014). *Atmospheric Environment*, *92*(C), 97–103.

Other non PILS based systems - Impinger based system QUT-HKCityU



Conclusion

- Most systems still in development phase with only 1 of them used as a real time monitor (Clarkson Uni).
- Large problems with auto-oxidation of the chemical probes especially DCFH.
- DTT very complicated to be used.

PERSONAL ROS SENSORS

Approach

FREE RADICAL CHEMISTRY BIOTECHNOLOGY
an ARC Centre of Excellence

Synthesis of novel Profluorescent Nitroxide probe molecules

Cooperative Research Centre for Polymers
Solutions for a better world

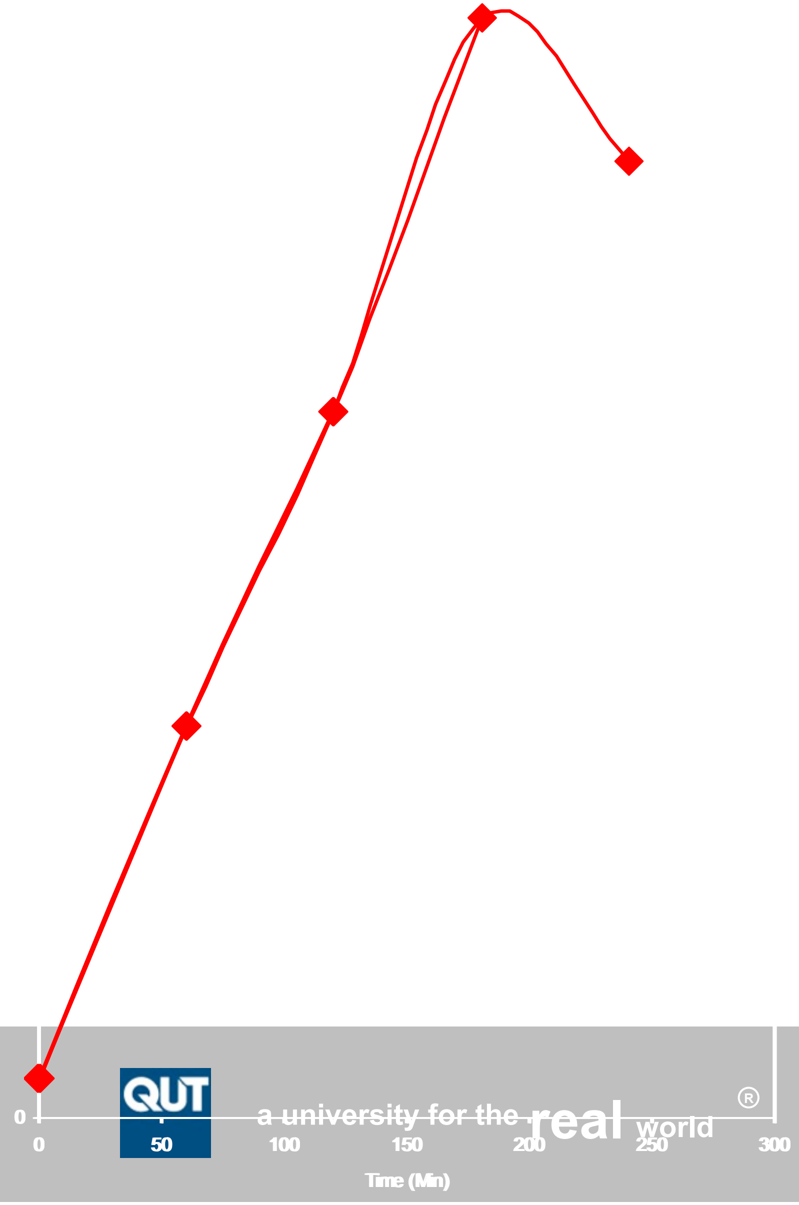
Embedding PFN probes in polymer matrices

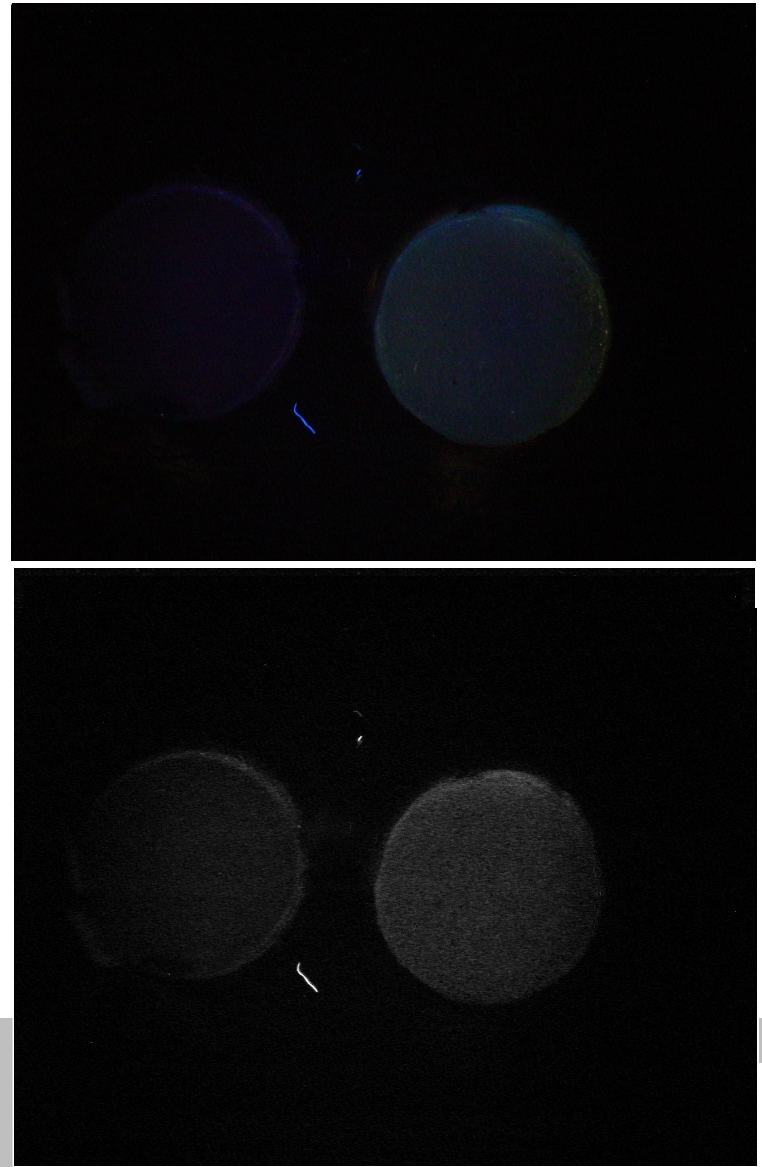
QUT International Laboratory for Air Quality and Health
ihbi
Institute of Health and Biomedical Innovation

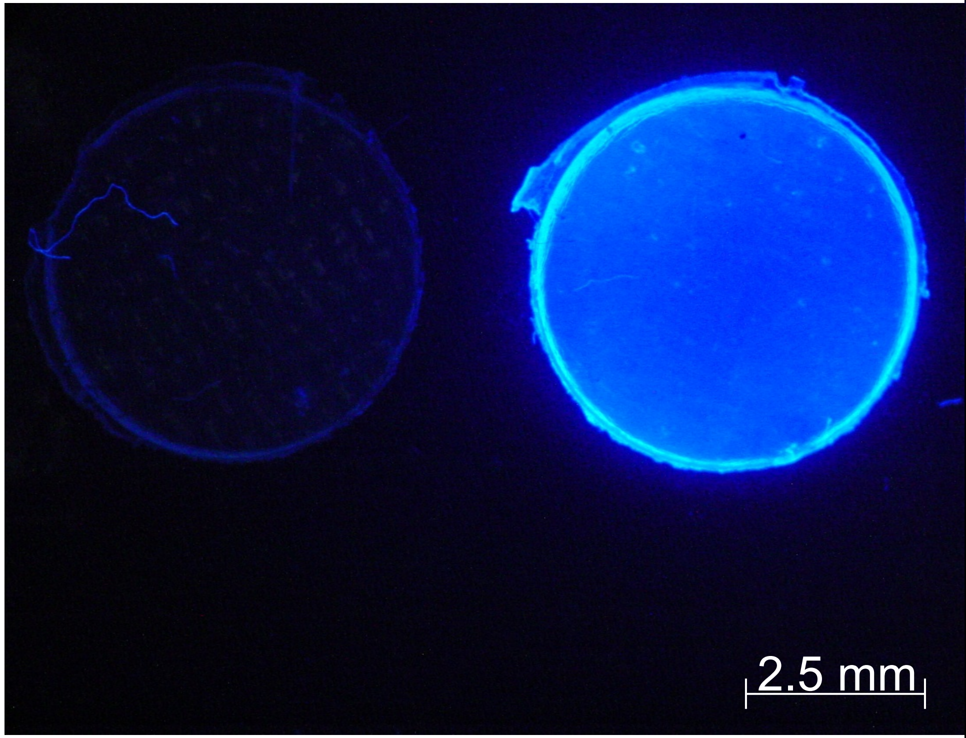
Implementation of the sensing technology in a portable personal sampler

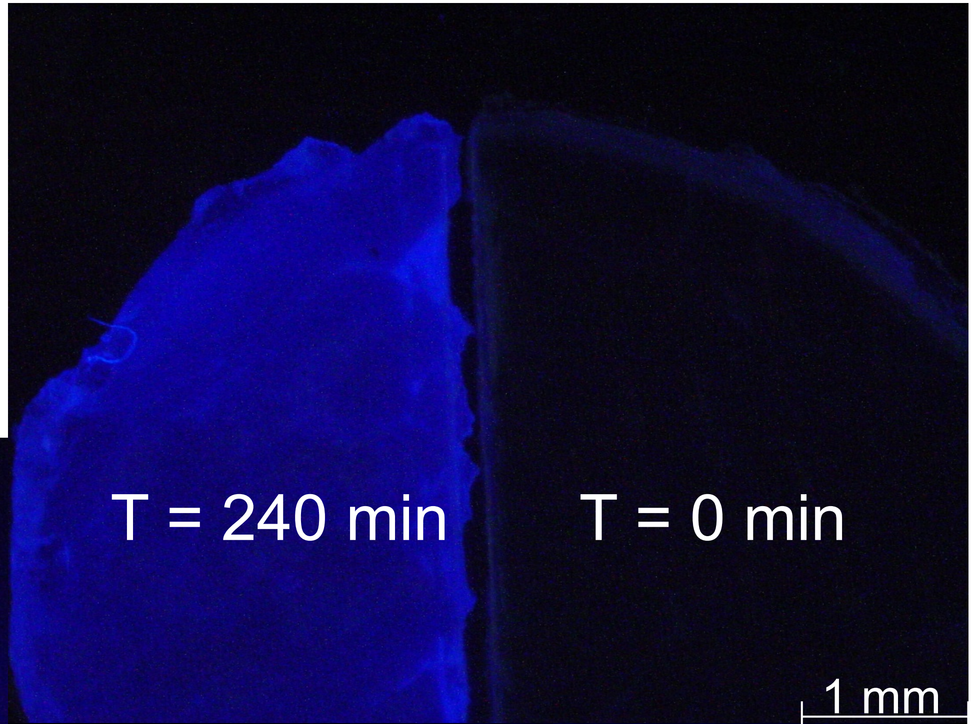
CURRENT TECHNOLOGY

Embedding PFN's into polymers for polymer degradation studies


0
0
50
100
150
200
250
300
Time (Min)

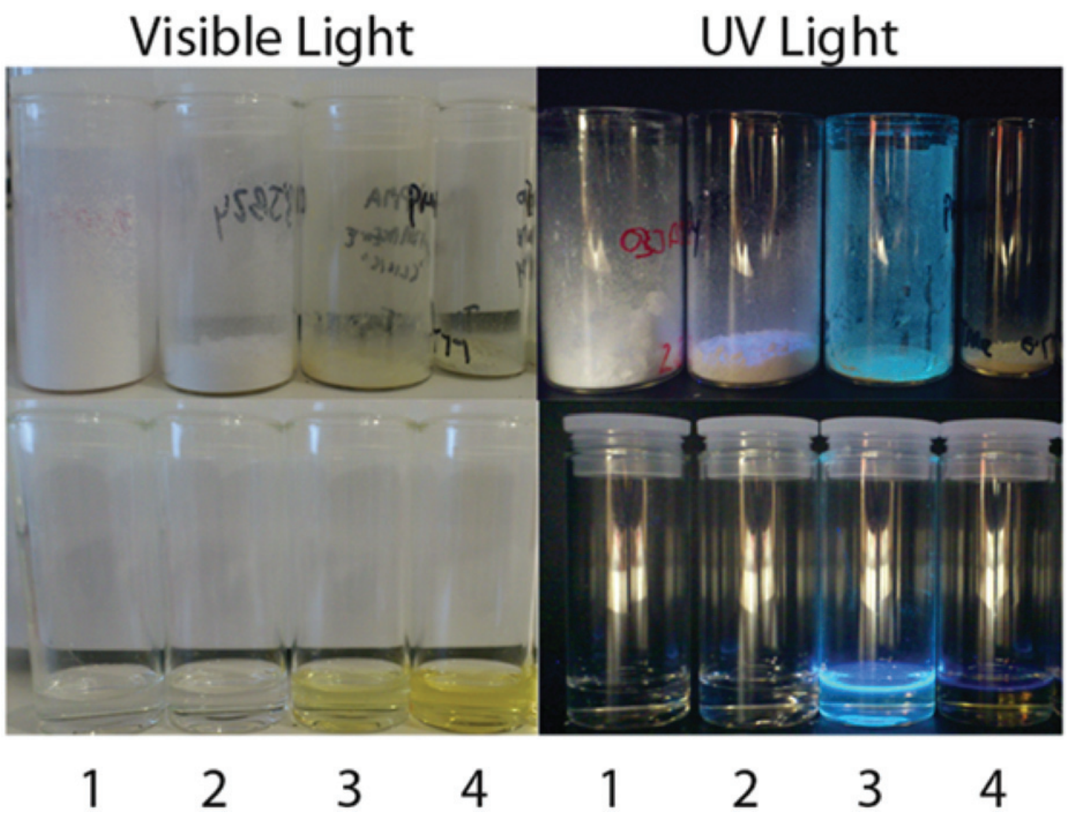



2.5 mm


T = 240 min
T = 0 min
1 mm


QUT
2.5 mm

Imbeding PFN's into polymers



Conclusion

- Technology still in development
- Problems of auto-oxidation of PFN's still not overcome
- Still not sufficient sensitivity for atmospheric levels of ROS.

Acknowledgment

- Organisers for the invitation to preent
- Funding:
  - Australian Coal Association Research Program
  - Australian Research Council
  - Institute for Future Environments

Thank you

Zoran Ristovski's group at ILAQH