WG4: Experimental methods applicable in research of flow and aerosol deposition in human airways and their replicas

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October 2019
SimInhale WG4: Advanced imaging, patient monitoring and delivery verification

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Role of WG4 within the project:

• Perform experimental measurements of air flow, aerosol flow and deposition in human lungs

• Close collaboration with WG3 (Computer simulations as a horizontal enabling technology: delivery, deposition and lung-tissue/particles interaction)
Current role of experimental methods

- Ever more often supplement computational simulations (CFD/CFPD)
- CFPD can predict local aerosol deposition in unbeatable resolution
- Significant differences among distinct methods of calculation emphasize the need for highly precise experimental data in order to specify boundary conditions and for validation purposes
In vivo vs „in replicas“

Repeatability vs Simplification

- Selection of a replica
- Brno lung model

https://timedotcom.files.wordpress.com/2014/11/lung-xray.jpg
Classification of experimental methods

1) Point-wise and planar methods for velocimetry in the airways

2) Classic methods for the measurement of the regional distribution of inhaled particles

https://www.dantecdynamics.com/images/

https://www.thomassci.com/

https://scales-measuring.com/
Classification of experimental methods

3) Standard medical imaging methods applicable to the measurement of the regional aerosol distribution

4) Emerging and nonconventional methods
1) Point-wise and planar methods for velocimetry in the airways

Constant Temperature Anemometry

- Wheatstone bridge compensates the cooling effect of the flowing fluid on the probe while maintaining a constant wire temperature.
- The current flowing through the wire creates heat, which is equal to the heat losses to the fluid. After the change of velocity, a servo amplifier immediately changes the current to the wire to keep the bridge in balance.
- The voltage drop across the sensor is proportional to the velocity of the fluid flow.

Selected advantages of CTA are:
- Fast frequency response: measurements up to several hundred kHz possible
- No seeding is required
- Measures velocity magnitude and fluctuations, also turbulent characteristics possible
- Relatively cheap

The main drawbacks of HWA are:
- Intrusive technique — disturbs the velocity field
- Insensitive to a reversal of flow direction
- Fragility — probe breakage and burn out
- Calibration required
### Laser Doppler based methods

A laser beam is split into two coherent beams that intersect symmetrically. The beams interfere and generate a series of parallel planes of light and darkness (fringes).

Any particles that are present in the flow scatter the light as they pass through the fringes.

In order to distinguish in which direction the particle travels, the frequency of one of the beams is shifted, so that the fringe pattern “rolls” with a known velocity.

A receiving lens projects the fringe patterns scattered by the particle onto a photodetector which produces a “Doppler burst” signal with a Doppler frequency. This is proportional to the velocity component.

### Selected advantages:
- high spatial and temporal resolution
- non-contact measurement; affordable price,
- highly accurate, directional sensitivity, suitable for unsteady reversal flows (as in airways),
- PDA variant adds the particle sizing capability,
- calibration not required

### Disadvantages:
- optical access required
- slow scanning technique, traversing a point matrix to provide planar or volumetric information
- indirect measurement technique, since it measures the velocity of tracer particles,
Example of application of Phase Doppler Anemometry – Brno lung model
Particle image velocimetry

- Two short light pulses with a distinctive time delay are emitted and two images of the illuminated flow are captured by a camera.
- Within the delay $\Delta t$ between the two images, particles move by a certain distance. Images are subdivided into small interrogation windows.
- Particle image patterns from the images are compared by cross correlation.
- A shift in each of the x- and y-directions is calculated forming a displacement vector. Thus a velocity vector can be determined for each interrogation window.

Selected advantages:
- 2D and 3D information of the whole flow field can be obtained simultaneously
- Nonintrusive measurement method - no flow disturbances
- High spatial and temporal resolution possible, depending on camera and light source

Disadvantages:
- Refractive index matching is required
- Use of small particles often requires use of high-intensity light sources (class 4 lasers)
- Reduced quality of results in high shear flows
- Tracer particles of special features (size, density,...) needed.
2) Classic methods for measurement of the regional distribution of inhaled particles

Measurements of concentrations using particle counters, spectrometers or photometers

- The usual experimental setup includes a source of particles, an in-line aerosol concentration measuring instrument, a flow rate measurement device, and a set of valves allowing the intake to switch between clean air and aerosol.
- The volunteer inhales a well-characterized monodisperse aerosol out of an aerosol generator through a mouthpiece, and then exhales.

\[ TDF = \frac{(C_{in,ave} \times \text{inhaled volume} - C_{out,ave} \times \text{exhaled volume})}{C_{in,ave} \times \text{inhaled volume}} \]

Advantages:
- no radioactivity involved,
- no simplification of the reality,
- can provide diagnostic indices.

Disadvantages:
- complicated control of boundary conditions (to be temporarily stable and inter and intra-subject invariable),
- difficulty with obtaining the geometry and boundary conditions for CFD.
Microscopy

- Mostly used with fibres.
- The fibres are dispersed, usually using a fluidized bed generator, treated to equalize the electric charge and mixed with inspiratory air.
- Then the fibres enter the model of lungs with coated walls, where a certain fraction deposits. The rest of the fibres are then collected on output filters.
- The fibres deposited in the model are consequently either counted directly in situ using the microscope (Myojo, 1987, 1990), or washed out, filtrated, and counted on the filter (Belka et al., 2016).


Selected advantages:
- simple and relatively cheap method,
- no radioactivity needed,
- can provide diagnostic indices.

Disadvantages:
- relatively high uncertainty,
- time-consuming,
- applicable only on replicas.
Example of application of microscopy – Brno lung model

Preparation of samples

IPA
Analysis of samples

• **Output filters**

  Suitable for automated analysis using image processing algorithm

• **Samples prepared by rinsing of the model segments**

  Fibers were counted manually
Image analysis:

a) original image and its histogram; b) image and histogram after Adaptive Contrast Control application; c) image and histogram after adaptive radial convolution, $T$ denotes threshold; d) segmented image; e) image with identified fibers

Results

\[ DF = \frac{\text{number of deposited fibers in a specific region}}{\text{number of fibers entering the oral airways}} \]

Gravimetry and Fluorometry, GC-MS

- The term gravimetry refers to a method based on weighing filters or collecting plates that contain deposited aerosol particles.
- The term fluorometry refers to those methods that are based on fluorescence measurements on the samples collected by washing out the segments of airways.
- The most common setup consists of the particle generator or disperser, a charge equilibrator, an aerosol-air mixer, a replica of lungs with connected output filters and a vacuum pump. Then, for gravimetry, either the whole replica or its parts are washed out, the resulting dispersion is filtrated and the filters analyzed.

**Selected advantages:**
- simple and relatively cheap methods,
- both routinely used in many laboratories.

**Disadvantages:**
- low spatial resolution (especially for gravimetry).
3) Standard medical imaging methods applicable to the measurement of the regional aerosol distribution

**Nuclear imaging methods**

- Mostly in vivo, several applications in replicas
- Based on determination of the spatiotemporal distribution of a radionuclide tracer
- Gamma ray-emitters or positron emitters (which ultimately result in the emission of gamma rays) are suitable radioisotopes for this purpose - high-energy gamma rays can travel through biological tissues without significant scatter or attenuation.
- The gamma rays can be detected and quantified using specific instrumentation and tomographic reconstruction algorithms.

When the nucleus of an atom has an excess of energy ➔ radioactive decay. The nucleus reaches a lower-energy state while emitting radiation in the form of alpha particles, beta particles (electrons or positrons), gamma rays or conversion electrons.
SPECT relies on the detection of gamma rays from radioactive decay of single photon-emitting radionuclides.

- Technetium-99 is the most common emitter (half-life of about 6 h)
- SPECT scanners consist of gamma-ray detection module(s) and collimator(s). The core of the detection module is a scintillation crystal which absorbs the energy of incident gamma rays and re-emits a flash of light, which is subsequently detected by a photo-electronic system.

The location in the crystal and the intensity of the flash of light are recorded. The collimator stops all the rays that do not reach the detector in a given direction. It forms a projected image of the radioisotope distribution on the surface of the scintillation crystal.
An emitted positron interacts with other charged particles and loses kinetic energy while transiting a random path. When most of this energy has been lost, the positron annihilates with an electron of a surrounding atom, resulting in the emission of a pair of gamma rays travelling in directions 180° apart.

The distance between the locations where disintegration and annihilation take place is called positron range (few mm in water).

Two photons detected almost simultaneously by two detectors are assumed to arise from one single annihilation.

PET scanners do not need collimators.

**Selected advantages:**
- Reasonable resolution and sensitivity
- Non-destructive
- No optical access needed
- Quantitative information available (PET)
- Available in medical and research centres

**Disadvantages:**
- Lack of anatomical information ➞ (hybrid scanners)
- Reaching the limits of resolution (mm-scale)
- Motion effects
- Quantification of images is difficult (SPECT)
- Stability of radiotracer – detachment of the radiotracer to the parent particle
Example of application of PET – Brno lung model
Proton MRI

Conventional MRI is based on the principle that atomic nuclei can absorb and emit electromagnetic energy when manipulated using an external magnetic field.

First, a strong magnetic field is applied to align the ‘spin’ of protons in hydrogen atoms, which are found in water molecules within the body. A radio frequency signal is then used to resonate the atoms, and the relaxation signal is measured by conductive coils placed around the patient.

Parameters of the magnetic field pulse sequence can be varied and hence different contrasts can be acquired between various tissues due to the relaxation properties of the hydrogen atoms in different tissues.

Selected advantages:
- No radioactivity (suitable for paediatric patients),
- Both flow velocity and aerosol distribution can be measured.

Disadvantages:
- Trace marker needed (for deposition studies),
- Long imaging time (low temporal resolution),
- Low differences in contrast between lung parenchyma and air,
- High doses of contrast agents needed.
4) Emerging and nonconventional methods

**Hyperpolarized gas MRI**

Conventional MRI: the weak signal intensity coming from the low density of air-filled lung parenchyma that results in low proton density

- Avoided by inhalation of high-contrast hyperpolarized noble gases ($^3$He or $^{129}$Xe).
- Normally, the gas polarization is zero, which means that half of the nuclear spins are pointed up, along the magnetic field, and half are pointed down.
- An application of a strong magnetic field would cause the upward spin to be slightly more frequent. However, in hyperpolarization, we strive to induce a situation where almost all spins are in one direction.
- It is carried out by flipping the down spin of the rubidium electrons up by absorbing angular momentum from laser photons, which causes their outer-shell valence electrons to become spin-polarized.

**Selected advantages:**

- Both flow field and particle deposition can be measured,
- Can be combined with SPECT and PET (for simultaneous measurements).

**Disadvantages:**

- Complex infrastructure and highly skilled personnel needed,
- Low resolution for flow measurement compared to PIV.
Optical Coherence Tomography (OCT)

- Based on the white light interferometry with near-infrared wavelength.
- The source of the light is a laser from which the light travels to a fibre coupler where it is divided into two parts, one part is reflected from a reference mirror, while the other part is scattered and partly reflected at the sample.
- The interfering light is recorded by a detector as a function of wavelength.
- There are several variants of OCT which differ in the way they analyse the recorded spectra and consequently in the achievable resolution and speed.

Selected advantages:
- fast 3D information without ionizing radiation,
- resolution in the µm range,
- Doppler-Information without additional hardware,
- only one optical port needed

Disadvantages:
- difficult marking of particles,
- optical access needed,
- depth range limited to some mm, maximal few cm at lower resolution.
Phase Contrast X-ray Imaging (PCXI)

- Unlike the conventional x-ray imaging which captures attenuating properties of structures, the PCXI is sensitive to the refractive and scattering properties of the sample.
- Attenuation of the signal by the sample causes a reduction in the amplitude of the incident x-ray waves which results in reduced intensity at the image detector.
- Soft tissues are not clearly seen in a conventional image, as they attenuate weakly. However, if an x-ray wavefield passes through a material with different refractive properties, a difference in the phase of the x-ray wavefield is introduced.
- PCXI is sensitive to these phase shifts and hence provides much better visualization of the soft tissues.

Selected advantages:
- high spatial resolution (down to 1 µm),
- high temporal resolution (up to 1000 Hz),
- 3D visualisation possible,
- possible in vivo.

Disadvantages:
- High radiation dose,
- signal from treatment not easily separated from the background in the way seen with nuclear imaging methods.
Example of application of PCXI

In vivo Dynamic Phase-Contrast X-ray Imaging using a Compact Light Source
Regine Gradl, Martin Dierolf, Benedikt Günther, Lorenz Hehn, Winfried Möller, David Kutschke, Lin Yang, Martin Donnelley, Rhiannon Murrie, Alexander Erl, Tobias Stoeger, Bernhard Gleich, Klaus Achterhold, Otmar Schmid, Franz Pfeiffer & Kaye Susannah Morgan
Scientific Reports, vol. 8, Article number: 6788 (2018)

„The motion of murine lungs during a slow breath cycle … The red dotted outline, fixed at the start of the breath, highlights the changes in the shape of the lung during inhalation. Panels (A–C) show the first 3 s of the whole breath cycle (8 s)... Panel (D) shows the corresponding X-ray velocimetry map during inhalation. As predicted, the largest movement is located at the lung periphery, as indicated by the warm end of the spectrum colour map used to display vector length. The maximum displacement vector length in (D) is 56 px."
<table>
<thead>
<tr>
<th>Technique</th>
<th>Spatial / time resolution</th>
<th>Quantities acquired / uncertainties</th>
<th>Cost / availability</th>
<th>Requirements</th>
<th>Special features</th>
<th>Calibration / adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HWA</td>
<td>1 mm / &lt;100 kHz</td>
<td>u (min. ±0.03 m/s; 5%) / 1C–3C</td>
<td>4 k€ for a single channel device/common in fluid mechanics</td>
<td>Inserting holes</td>
<td>Equidistant sampling, intrusive measurement</td>
<td>Daily / daily</td>
</tr>
<tr>
<td>LDA/PDA</td>
<td>0.1 mm / &lt;100 kHz</td>
<td>u (±1%); D (±0.5 µm); c (±20%)</td>
<td>40–80 k€ per channel / common in fluid dynamics</td>
<td>Optical access, seeding</td>
<td>Random time sampling, on-line</td>
<td>No / daily</td>
</tr>
<tr>
<td>PIV/PTV</td>
<td>&gt;1 µm (for µ-PIV) / &lt;10 kHz</td>
<td>u, 2C–3C / 0.2 px</td>
<td>80–200 k€ depending on PIV-type</td>
<td>Optical access, refractive index matching, seeding</td>
<td>Stereo PIV, Tomo-PIV, µ-PIV, scanning-PIV, endoscopic PIV</td>
<td>For each measurement</td>
</tr>
<tr>
<td>Concentration meas.</td>
<td>Airway generation / single breath</td>
<td>c (±10%)</td>
<td>About 20 k€</td>
<td>Trained volunteers / lung replica</td>
<td>Simple, easily available</td>
<td>Annually / per experiment</td>
</tr>
<tr>
<td>Microscopy</td>
<td>1 mm to generation / per breath</td>
<td>c (±30%)</td>
<td>3.5 k€ per equipped microscope/common in environmental sciences</td>
<td>Separable replica of airways, filter preparation equipment</td>
<td>Simple, time-consuming, established methodology</td>
<td>Monthly; regular intra and inter-laboratory checks</td>
</tr>
<tr>
<td>Gravimetry / Fluorometry</td>
<td>Bifurcation, several cm²</td>
<td>c (±10%)</td>
<td>3 k€ (laboratory balance), units of k€ for fluorometer</td>
<td>Separable replica or volunteer (for fluorometry)</td>
<td>Simple, low resolution or reduced extent</td>
<td>Annually / per experiment</td>
</tr>
<tr>
<td>Technique</td>
<td>Spatial / time resolution</td>
<td>Quantities acquired / uncertainties</td>
<td>Cost / availability</td>
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<td>Special features</td>
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<td>PET</td>
<td>1–5 mm</td>
<td>CPS</td>
<td>Expensive, human scanners &gt; 1 M€, radiochemistry facilities required.</td>
<td>Species to be tracked labelled with a positron emitter</td>
<td>In vivo, 4D (time + space), minimally invasive</td>
<td>Periodically (e.g. monthly)</td>
</tr>
<tr>
<td>SPECT/CT</td>
<td>Typically 5–10 mm in clinical scanners</td>
<td>CPS</td>
<td>Cheaper than PET, most hospitals have SPECT, radiochemistry facilities required.</td>
<td>Species to be tracked labelled with a gamma emitter</td>
<td>In vivo, 3D, minimally invasive</td>
<td>Periodically (e.g. monthly)</td>
</tr>
<tr>
<td>³He MRI</td>
<td>u, 3C, ±6.4%; ±25 mm/s</td>
<td>Low availability</td>
<td>MRI with broadband amplifier; gas polarizer; dedicated chest coil; ³He or ¹²⁹Xe supplies;</td>
<td>3D flow dynamics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCXI</td>
<td>1 µm / 100 Hz</td>
<td>X-ray image sequence → u</td>
<td>Access to set-ups available via peer-reviewed application, without cost</td>
<td>X-ray absorbing or phase-shifting features</td>
<td>PIV of lung motion, 2D, 3D (time/space), 4D (time+space), individual particle motion</td>
<td>No</td>
</tr>
<tr>
<td>OCT</td>
<td>1–10 µm / single depth line ~1–10 µs, plane ~1 ms, volume 0.1 s</td>
<td>u, c</td>
<td>50–200 k€</td>
<td>Optical access, seeding</td>
<td>In vivo, 3D</td>
<td>No / rare</td>
</tr>
</tbody>
</table>
Conclusions

• Role of experimental methods in combination with CFPD
  ➢ Acquisition of the lung geometry, defining the boundary conditions, validating the local flow velocities and particle deposition possible to predict localized deposition and patient-specific or disease-related features

• The crucial step for the selection of a suitable combination of experimental data or instrumentation is acquiring and reproducing the lung geometry.
  ➢ When comparing distinct methods of CFPD calculations, it is necessary to use the identical lung geometry with sufficient experimental data to evaluate success.

• After the validation in a standard lung geometry model, the simulation could be extended and compared to in vivo data to verify agreement with real physiological processes in general terms.
Thank you for your attention

Brno, the Czech Republic

The authors would like to acknowledge the support provided by the COST-European Cooperation in Science and Technology, Action MP1404: Simulation and pharmaceutical technologies for advanced patient-tailored inhaled medicines (SimInhale).