# Charting the human lung with inhaled nanoparticles

## Development of AiDA - a new diagnostic technique for respiratory disease

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UNIVERSITY

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Charting the human lung with inhaled nanoparticles
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Changes to the smallest structures of the distal lung are often the first sign of severe respiratory disease, but to date methods to detect and quantify these changes are limited. Chronic obstructive pulmonary disease (COPD) is currently estimated to be the third most common cause of death worldwide, but is to a large extent under-diagnosed, especially at early stages. This thesis explores a new method for examining the lung by measurements of lung deposition of airborne nanoparticles, with possible potential to be developed into a diagnostic method for detection of changes typical for early COPD. The technique is called Airspace Dimension Assessment with nanoparticles (AiDA).

An instrument for lung deposition measurements was constructed and characterized on healthy subjects. The instrument produces a test aerosol containing monodisperse nanoparticles, which can be administered to subjects in inhalation experiments. The particle concentration in the inhaled test aerosol and in exhaled air from determined volumetric sample depths can be determined after controlled residence times in the lung. By comparing inhaled and exhaled concentrations, the deposition efficiency of the nanoparticles can be determined in a single breath. The instrument is, to our knowledge, currently the only instrument with this capability.

Particle deposition was measured on healthy individuals and individuals with COPD. It was found that particle deposition was decreased for subjects with COPD, and this was interpreted as an effect of enlarged airspaces, pulmonary emphysema. This shows that particle deposition measured with the instrument reflects subject specific lung properties.

A theory was developed for interpretation of the relation between measurements of lung deposition and properties of the lung. Nanoparticles deposit almost exclusively by diffusion in the distal lung. By measuring the half-life time of particles in the lung, effective airspace radii can be estimated which reflect lung geometry. Repeated measurements on three subjects with 15 -18 months between the measurements showed that the precision was on average ±7 µm. Particle deposition during the dynamic part of the breathing manoeuvres may also provide information about mainly the small conducting airways.

The theory was evaluated by measurements on healthy subjects. It was shown that both the derived effective airspace radii and the particle deposition during the dynamic phase of the measurement were in agreement with expectations from the developed theory. It was also shown that the parameters correlated with subject characteristics and physiological measurements.

An AiDA measurement protocol was developed which can be performed by most subjects in less than 15 minutes. AiDA measurements have been performed on more than 1000 subjects. This exceeds the total number of subjects included in previously published studies on lung deposition of nanoparticles. Preliminary results show that the technique fulfils several criteria for an efficient diagnostic technique. Preliminary data also indicates that the technique may give relevant medical information about the distal lung not easily obtainable by other means, and thus may be considered as a feasible future diagnostic tool for the lung.

Key words: Aerosol, lung, lung deposition, respiratory tract, nanoparticles, ultrafine particles, airspace dimension assessment, AiDA, Chronic obstructive pulmonary disease, COPD

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*For breath is life, and if you breathe well you will live long on earth.*

Sanskrit Proverb

# Contents

# Abstract

Changes to the smallest structures of the distal lung are often the first sign of severe respiratory disease, but to date methods to detect and quantify these changes are limited. Chronic obstructive pulmonary disease (COPD) is currently estimated to be the third most common cause of death worldwide, but is to a large extent under-diagnosed, especially at early stages. This thesis explores a new method for examining the lung by measurements of lung deposition of airborne nanoparticles, with possible potential to be developed into a diagnostic method for detection of changes typical for early COPD. The technique is called Airspace Dimension Assessment with nanoparticles (AiDA).

An instrument for lung deposition measurements was constructed and characterized on healthy subjects. The instrument produces a test aerosol containing monodisperse nanoparticles, which can be administered to subjects in inhalation experiments. The particle concentration in the inhaled test aerosol and in exhaled air from determined volumetric sample depths can be determined after controlled residence times in the lung. By comparing inhaled and exhaled concentrations, the deposition efficiency of the nanoparticles can be determined in a single breath. The instrument is, to our knowledge, currently the only instrument with this capability.

Particle deposition was measured on healthy individuals and individuals with COPD. It was found that particle deposition was decreased for subjects with COPD, and this was interpreted as an effect of enlarged airspaces, pulmonary emphysema. This shows that particle deposition measured with the instrument reflects subject specific lung properties.

A theory was developed for interpretation of the relation between measurements of lung deposition and properties of the lung. Nanoparticles deposit almost exclusively by diffusion in the distal lung. By measuring the half-life time of particles in the lung, effective airspace radii can be estimated which reflect lung geometry. Repeated measurements on three subjects with 15 -18 months between the measurements showed that the precision was on average ±7 μm. Particle deposition during the dynamic part of the breathing manoeuvres may also provide information about mainly the small conducting airways.

The theory was evaluated by measurements on healthy subjects. It was shown that both the derived effective airspace radii and the particle deposition during the dynamic phase of the measurement were in agreement with expectations from the developed theory. It was also shown that the parameters correlated with subject characteristics and physiological measurements.

An AiDA measurement protocol was developed which can be performed by most subjects in less than 15 minutes. AiDA measurements have been performed on more than 1000 subjects. This exceeds the total number of subjects included in previously published studies on lung deposition of nanoparticles. Preliminary results show that the technique fulfils

several criteria for an efficient diagnostic technique. Preliminary data also indicates that the technique may give relevant medical information about the distal lung not easily obtainable by other means, and thus may be considered as a feasible future diagnostic tool for the lung.

# Populärvetenskaplig sammanfattning

Uppskattningsvis var fjortonde svensk kan vara drabbad av en av världens dödligaste sjukdomar, utan att ens veta om det. Syftet med denna avhandling är att utforska en ny diagnosmetod som skulle kunna upptäcka denna sjukdom på ett tidigt stadium. Detta är viktigt eftersom det gör att behandling kan sättas in som begränsar de skador som sjukdomen orsakar. Sjukdomen i fråga är kronisk obstruktiv lungsjukdom (KOL) och metoden kallas Airspace Dimension Assessment (AiDA), och bygger på att mäta andelen nanopartiklar som deponeras i lungan under kontrollerade andningsmönster.

Vid denna avhandlings produktion bedöms KOL vara världens tredje vanligaste dödsorsak, men bara en liten del av dem som drabbats är idag korrekt diagnosticerade. Detta beror på att dagens diagnosmetoder för tidig KOL är begränsade, vilket är ett allvarligt problem. Sjukdomen är nämligen obotlig och en tidig diagnos är kritisk för att sätta in behandling som bromsar sjukdomsförloppet och ger bibehållen livskvalitet.

KOL kan förenklat sägas bestå av två huvudkomponenter: Bronkiolit och emfysem. Bronkiolit är en kronisk inflammation som huvudsakligen drabbar de små ledande luftvägarna. Emfysem drabbar vävnaden längre ut i lungan som huvudsakligen består av alveoler (lungblåsor). Emfysem innebär att alveolernas väggar brister och att de gradvis smälter ihop och bildar större hålrum. Proportionerna mellan bronkiolit och emfysem varierar mellan olika patienter vilket gör att KOL kan vara svårt att diagnosticera. Särskilt emfysem, som är ett mycket allvarligt tillstånd, är svårt att upptäcka tidigt.

Diagnos av KOL är idag oftast baserad på spirometri, som innebär att man mäter lungvolymer och flöden vid andning. Detta kan kompletteras med mätning av lungans diffusionsegenskaper för kolmonoxid ($D_{L,CO}$) samt i vissa fall även med datortomografi av lungorna. Spirometri är ganska enkelt att utföra och kan visa att någonting inte står rätt till med lungorna, men kan inte riktigt visa orsaken till besvären. Datortomografi är dyrt, kräver avancerad utrustning och tolkning av en specialist, och innebär dessutom att patienten utsätts för strålning. $D_{L,CO}$ påverkas av många olika faktorer utöver de förändringarna som förekommer vid KOL. Sammanfattningsvis finns det ett stort behov av nya, enkla och effektiva diagnosmetoder.

Den grundläggande tanken bakom AiDA är till synes enkel. Vid varje andetag vi tar andas vi in partiklar som genom olika mekanismer deponeras i våra lungor. De allra minsta partiklarna (<100 nm) deponeras i lungorna nästan uteslutande genom diffusion, en process som är beroende av partiklarnas diffusionshastighet, deras uppehållstid i lungan och deras avstånd till tillgängliga ytor. Om man väljer ut partiklar med väldefinierad storlek kommer deras diffusionshastighet också att vara väldefinierad och känd. Om man mäter hur stor del av dessa partiklar som deponeras i lungan under en bestämd tid så borde man kunna dra slutsatser om de genomsnittliga avstånden i luftrummen. Att göra denna typ av mätning

skulle kunna ge information om förekomsten och graden av emfysem. Emfysem innebär ju att avstånden mellan ytor i lungvävnaden ökar. Om man dessutom undersöker hur stor andel av partiklarna som deponeras då partiklarna rör sig genom de ledande luftvägarna är det inte långsökt att man kan få information om denna del av lungan också. Förträngda luftvägar och andra förändringar som uppkommer vid bronkiolit kan antas öka deponeringen av partiklar. Denna effekt är dock något mer komplicerad.

Denna avhandling inkluderar fyra arbeten som tillsammans utgör en bas för utvecklingen av AiDA. Artikel I behandlar den teoretiska bakgrunden för AiDA och dess förväntade fördelar och begränsningar. Bland annat visas att det mått man får på lungans dimensioner är ett kvadratiskt medelvärde på effektiva diffusionsavstånd i lungan. Ett sådant medelvärde påverkas starkare av större avstånd än av små, vilket är en fördel om man letar just efter förstorade dimensioner, som vid emfysem. Artiklarna II-IV beskriver olika kliniska studier, som bekräftar den teori som presenteras i artikel I.

I artikel II beskrivs hur ett instrument kan konstrueras som kan mäta lungdeponering av nanopartiklar med mycket hög precision i ett andetag. Det konstruerade instrumentet är i dagsläget det enda instrument som kan göra detta. Precisionen på mätningarna i instrumentet visade sig vara 26-50 gånger större än individvariationen för en liten grupp friska individer. Detta visar att mätningarna ger information på individnivå. Artikeln visar också att partikeldeponeringen varierar med uppehållstiden i lungan och partikelstorleken på ett sätt som stämmer med partikeldeponering genom diffusion, och att flödeshastigheten vid in och utandningen har liten betydelse. Detta innebär att man kan genomföra mätningar med ett ganska fritt andningsmönster jämfört med tidigare tekniker. Mängden nanopartiklar som behöver användas för en mätning är mycket liten och motsvarar ungefär den mängd vi får i oss när vi drar ett andetag luft i en svensk småstad, eller en miljondel av att röka en cigarett.

I artikel III jämförs friska individer med individer diagnosticerade med KOL. Studien visar att AiDA-mätningar även går att genomföra på individer med svår lungsjukdom, och att partikeldeponeringen skiljer sig mellan individer med KOL och friska individer. Detta stämmer överens med teorin som presenteras i artikel I. Det visas också att partikeldeponeringen korrelerar med lungfunktionstester, speciellt för den sjuka gruppen. Detta är viktigt eftersom det visar att det kan finnas en klinisk tillämpning för AiDA. I artikel III visas också, inte helt oväntat, att partikeldeponeringen påverkas av hur djupt ner i lungan man mäter. Detta innebär att tekniken kan ha viss volymupplösning, och att man kanske kan få information om olika delar av lungan. Detta undersöks vidare i artikel IV där AiDA används för att undersöka inverkan av lungdjup, samt om man kan få information både genom att mäta vad som händer med partiklarna under andhållning och då de rör sig genom lungan vid in- och utandning.

Det visas i artikel IV att man genom teorin i artikel I och mätningar med instrumentet kan beräkna luftrummens dimensioner i olika delar av lungan och att resultaten stämmer ganska

väl överens med lungmodeller som baserats på analys av verkliga undersökta lungor. Upprepade mätningar på tre individer med upp till ett och ett halvt års mellanrum visade att dimensioner på luftvägarna kunde mätas med en precision på omkring 7 µm, vilket är tillräckligt för att också kunna identifiera mycket små avvikelser från det normala. Det visades också att det finns samband mellan mätningarna och forskningspersonernas ålder, längd, vikt och olika lungfunktionstester, även om det rörde sig om en ganska liten grupp av friska individer. Särskilt är sambandet med ålder intressant, då ålder har visats påverka lungans struktur. Ett särskilt viktigt och intressant resultat i artikel IV är att man genom att separera partikeldeponeringen som uppkommer då aerosolen passerar genom de ledande luftvägarna från den som uppkommer under andhållningen i den djupare lungan sannolikt kan få oberoende information om olika delar av lungan. Detta är helt i linje med teorin i artikel I.

Resultaten i de inkluderade artiklarna har lett till utvecklingen av ett kort och effektivt mätprotokoll för tekniken som kan genomföras på ungefär 15 minuter och ger en uppskattning både av storleken på luftrummen i den djupa lungan och på partikeldeponeringen då luften strömmar genom de ledande luftvägarna. Detta mätprotokoll har genomförts på ett stort antal forskningspersoner, och de preliminära resultaten är mycket intressanta.

Bland annat har det visat sig att mätningarna korrelerar med egenskaper som ålder, längd och vikt för forskningspersonerna och även med deras resultat på olika lungfunktionstester. De statistiska sambanden är förhållandevis säkra, men inte starka. Detta är vad man kan förvänta sig för en huvudsakligen frisk grupp.

Det har även visat sig att mätningarna med AiDA korrelerar med forcerad oscillationsteknik (FOT). FOT är en teknik som är baserad på mätning av hur ljudvågor interagerar med lungan och har på senare tid vunnit ökande intresse främst på grund av sitt enkla utförande och lovande resultat. Det finns dock aspekter av FOT som inte kan betraktas som fullständigt utredda och vissa av dessa skulle man kunna öka förståelsen av genom att jämföra med AiDA. Den preliminära analysen visar att partikeldeponeringen som mäts i de ledande luftvägarna, men inte i de djupare delarna av lungan med AiDA korrelerar tydligt med FOT. Resultaten antyder att FOT huvudsakligen ger signal från de ledande luftvägarna och inte från hela lungan, som man tidigare antagit.

Sammanfattningsvis visar de redovisade resultaten att AiDA har potential att utvecklas till en användbar teknik för att mäta olika lungegenskaper. Studierna bidrar även till en ökad förståelse av deponering av nanopartiklar i luftvägarna, inklusive data för lungdeponering av nanopartiklar på ett stort antal individer - fler än det totala antalet deltagare i alla liknande studier sammanlagt. Detta är ett område där tidigare experimentell data varit begränsad.

Avhandlingen presenterar grunden för en framtida utveckling av AiDA, men resultaten väcker även nya frågeställningar. Det återstår till exempel att mer ingående undersöka metodens känslighet och specificitet för olika lungsjukdomar, att göra tekniken mer lättanvänd och att utforska tillämpningsområden inom primär- och företagshälsovård. Teoretiska modeller för att analysera och tolka partikeldeponering i de ledande luftvägarna återstår också att utveckla. Det finns även utrymme för att förenkla och effektivisera teknologin som används för att genomföra mätningar med AiDA metoden.

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# List of symbols and abbreviations

ADAM = Aerosol-Derived Airway Morphometry
ADC = Apparent diffusion coefficient
AiDA = Airspace Dimension Assessment
Ax = Reactance area
COPD = Chronic Obstructive Pulmonary Disease
CFD = Computational fluid dynamics
CPC =Condensation Particle Counter
CT = Computed Tomography
$D$ = Diffusion coefficient
DEHS = Di(2-ethylhexyl) sebacate
$DF$ = Deposition Fraction
DMA =Differential Mobility Analyser
$D_{L,CO}$ = Diffusing capacity for carbon monoxide
EAD = Effective Airway Dimension
ERV = Expiratory Reserve Volume
EI = Emphysema Index
FOT = Forced Oscillation Technique
Fres= Resonant frequency
FVC = Forced Vital Capacity
$FEV_1$ = Forced Expiratory Volume in 1 second
GSD = Geometric Standard Deviation
$K_{CO}$ = Transfer coefficient of carbon monoxide
$LAV_{\%}$ = Low attenuation volume
LLN = Lower limit of normal
ICRP = International Commission on Radiological Protection
IOS = Impulse Osciollometry System
MLD = Mean lung density
MPPD = Multiple-Path Particle Dosimetry
MRI = Magnetic Resonance Imaging
$PD_{15}$ = 15th percentile lung density
PEF = Peak Expiratory Flow
PM = Particulate Matter (airborne)
PSL = PolyStyrene Latex

$R$ = Particle recovery

$r_{AiDA}$ = Effective airspace radius

$r_{AiDA(V)}$ = Effective airspace radius at nominal volumetric sample depth V

$R_0$ = Zero second recovery

$R_5$ = Respiratory resistance at 5 Hz

$R_{20}$ = Respiratory resistance at 20 Hz

$R_{korr}$ = Reported Recovery, with particle loss adjustments

$R_{rs}$ = Respiratory resistance

$R_{instrument}$ = Recovery, due to particle loss in instrument

RV =Residual Volume

S = Stopping distance

SCAPIS = Swedish CardioPulmonary bioImage Study

Stk = Stoke´s number

$t_i$ = Total time the aerosol passes through the instrument

$t_{1/2}$ = Particle half life time in the lung

TLC =Total Lung Capacity

$V_{LD}$ = Volumetric lung depth

$V_t$ = Terminal settling velocity

$V_s$ = Settling velocity

$V_{LD,R}$ = Normalized volumetric lung depth, $V_{LD}/V_i$

$V_T$ = Tidal Volume

$X_{rs}$ = Respiratory reactance

$X_5$ = Respiratory reactance at 5 Hz

$Z_{rs}$ = Respiratory impendance

# List of papers included in this thesis

**I.** **Do nanoparticles provide a new opportunity for diagnosis of distal airspace disease?**
Löndahl, J., **J. K. Jakobsson**, D. M. Broday, H. L. Aaltonen and P. Wollmer. Int J Nanomedicine, 2017, 12: 41-51.

**II.** **A new method for measuring lung deposition efficiency of airborne nanoparticles in a single breath**
**Jakobsson, J. K. F.**, J. Hedlund, J. Kumlin, P. Wollmer and J. Löndahl 2016 Nature Scientific Reports, 2016, 6: 36147.

**III.** **Altered deposition of inhaled nanoparticles in subjects with chronic obstructive pulmonary disease**
**Jakobsson J. K. F.**, Aaltonen H. L., Nicklasson H., Gudmundsson A, Rissler J., Wollmer P., Löndahl J., BMC Pulm Med, 2018. 18(1): p. 129.

**IV.** **Charting the human respiratory tract with airborne nanoparticles - Evaluation of the Airspace Dimension Assessment technique**
**Jakobsson J. K. F.**, Wollmer P., Löndahl J., 2018, Journal of Applied Physiology, (Accepted for publication in *Journal of Applied Physiology*, 4 September 2018).

# Author's Contributions to the papers

**I.** I made parts of the literature review and contributed to the discussions that shaped the paper. I performed some of the data analysis and wrote parts of paper.

**II.** I participated in the development of the instrument and finalized the experimental set-up. I took part in planning of the measurements, recruited the subjects, performed the measurements and analysed the data. I wrote the first version of the manuscript and finalised the manuscript together with the other authors.

**III.** I designed the study together with the other authors, performed the majority of the experiments, made the data analysis and wrote the first version of the manuscript and finalised the manuscript together with the other authors.

**IV.** I had a major role in the planning of the experiments, wrote the application to the ethical review board, recruited the subjects, performed the experiments, analysed the data and wrote the first version of the manuscript and finalised the manuscript together with the other authors.

# Publications not included in this thesis

## Peer-reviewed papers

Aaltonen, H. L., **Jakobsson, J. K.**, Diaz, S., Zackrisson, S., Piitulainen, E., Löndahl, J., & Wollmer, P. (2018). Deposition of inhaled nanoparticles is reduced in subjects with COPD and correlates with the extent of emphysema: proof of concept for a novel diagnostic technique. *Clin Physiol Funct Imaging*. doi:10.1111/cpf.12517

Aaltonen, H. L., Kindvall, S., **Jakobsson, J. K. F.**, Löndahl, J., Olsson, L. E., Diaz, S., Wollmer, P. (2018). Airspace dimension assessment with nanoparticles reflects lung density as quantified by MRI. *International Journal of Nanomedicine*, 13, 2989—2995. doi:https://doi.org/10.2147/IJN.S160331

Alsved, M., Civilis, A., Ekolind, P., Tammelin, A., Andersson, A. E., **Jakobsson, J.**, Svensson T., Ramstorp M., Sadrizadeh S., Larsson P., Bohgard M., Šantl-Temkiv T., Löndahl, J. (2018). Temperature-controlled airflow ventilation in operating rooms compared with laminar airflow and turbulent mixed airflow. *Journal of Hospital Infection*, 98(2), 181-190. doi:10.1016/j.jhin.2017.10.013

Ling, M. L., Wex, H., Grawe, S., **Jakobsson, J.**, Löndahl, J., Hartmann, S., K. Finster, T. Boesen, Šantl-Temkiv, T. (2018). Effects of Ice Nucleation Protein Repeat Number and Oligomerization Level on Ice Nucleation Activity. *Journal of Geophysical Research-Atmospheres*, 123(3), 1802-1810. doi:10.1002/2017jd027307

# Conference proceedings (selection)

**J. Jakobsson**, HL. Aaltonen, H. Nicklasson, S. Diaz, P. Wollmer, J. Löndahl (2018). *Characterization of Airspace Dimension Assessment with Nanoparticles (AiDA) on a Large Population and Relation to Anthropometry and Lung Function Parameters.* In: International Aerosol Conference, 2018, 2 September -7 September, St. Louis, United States of America.

M. Ling, H. Wex, S. Grawe, **J. Jakobsson**, S. Hartmann, J. Löndahl, K. Finster, T. Boesen, T. Šantl-Temkiv (2018). *Laboratory Studies on Mechanisms Behind Bacterial Ice-Nucleation Activity.* Invited. In: International Aerosol Conference, 2018, 2 September -7 September, St. Louis, United States of America.

J. Löndahl, **J. Jakobsson**, HL. Aaltonen, E Piitulainen, P. Wollmer. J. (2018). *Phenotyping of Chronic Obstructive Disease, COPD, from Deposition Fractions of inhaled Nanoparticles.* In: International Aerosol Conference, 2018, 2 September -7 September, St. Louis, United States of America.

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# Introduction

Breathing is essential for our existence. We can survive a few weeks without food and a few days without water, but only a few minutes without air. Even a slight impairment of this critical function can have a large impact on our quality of life. Small changes and abnormalities in the distal airspaces of the lungs is often the first stage of serious respiratory disease, however few techniques are available which are able to detect such changes, especially at an early stage. This thesis describes a putative new method with the intended use to detect, and perhaps even quantify, small alterations in the lungs by measurements of lung deposition of inhaled nanoparticles.

The air around us is full of particles. Even a moderate estimation shows that we inhale on average 100 000 000 000 particles on a normal day in a typical clean environment [1]. What happens to these particles? Some of them are just exhaled again. Other particles reach a surface somewhere in our respiratory tract, and deposit. What decides if an inhaled particle deposits or not? This depends on the properties of the particle, the way we breathe and the geometry of our lungs.

Nanoparticles are loosely defined as particles that have at least one dimension smaller than 100 nm. Particles of this size have several interesting properties, but the properties that are relevant for this thesis are the properties of nanoparticles when they are suspended in air. More specifically in the air that we inhale. Because of their small size and almost negligible weight, nanoparticles suspended in air behave much like enormous and very slow gas molecules [2]. They readily follow convective airflow resulting from our breathing and reach the distal lung, where they may deposit by diffusion. The high ability of nanoparticles to follow airflow, combined with the movement by diffusion, make it plausible that they can be used to examine the properties of the lungs, and possibly provide a new approach for diagnosis of one of the world's deadliest diseases, chronic obstructive pulmonary disease (COPD).

# The 3rd leading cause of death worldwide

Chronic obstructive pulmonary disease (COPD) is currently estimated to be the 3rd most common cause of death worldwide and increasing [3]. According to the most recent available data from the global health observatory, 3.2 million people died from COPD worldwide in 2015 [4] and it is estimated that 174 million people were suffering from the disease in 2016 [5]. Thus, COPD is currently estimated to be the 3rd most common cause of death worldwide and the prevalence is increasing [3]. In Sweden, it is estimated that 8 - 10% of the adult population, or 500 000 – 700 000 people, are affected by COPD, but the estimation is uncertain, as just a fraction of the affected are diagnosed, especially at the earlier stages of the disease [6, 7]. It is estimated that the number of deaths caused by COPD in Sweden is as high as 3000 on an annual basis [6], which can be compared to the annual deaths in traffic in Sweden for the last 5 years (2012-2017) which was on average 284 [8]. The management of COPD also result in large societal costs [7], thus the disease is not just a burden for the diseased induvidial, but for society as a whole. The ranking of the 10 most common causes of death worldwide is shown in Figure 1:

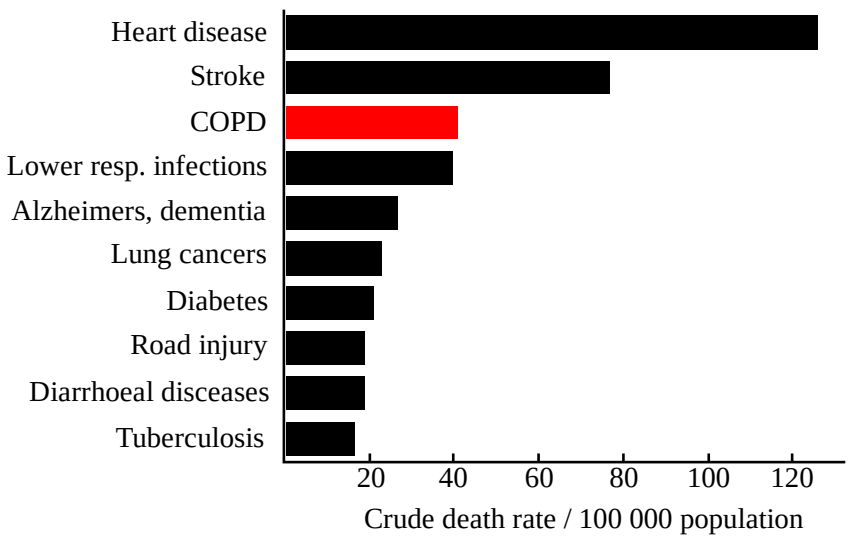


**Figure 1:**
The top 10 causes of death worldwide, Global Health Observatory data, 2016. COPD is ranked as the 3rd most common cause of death [3].

# The importance of efficient diagnostic techniques

COPD is a slowly progressive disease with irreversible damage to the lung and a poor long time prognosis. The initial symptoms are often vague, and can be overlooked, both by the patient and health care professionals. As the disease progresses, early mild symptoms, often experienced as frequent colds or shortness of breath, may not call for immediate attention. The progression of the disease is slow, and the patient usually adapts over time and therefore do not realize the severity of the condition until at a late stage. At this stage, the destruction of lung tissue is usually extensive, and the possibilities of effective treatment is limited.

Medical and physical treatment can help relieve the symptoms of COPD and slow down the progression of the disease, but there is no cure, and the efficiency of such treatment is dependent on the stage at which it is applied. Thus, an early diagnosis is critical for the patient outcome.

The most recognized risk factor for COPD is cigarette smoking [9], but also other forms of exposure to airborne pollutants, such as environmental and occupational exposure, have been identified as risk factors of increasing interest [10]. The most important action for COPD treatment for most patients is an immediate cessation of the hazardous exposure (smoking or other) and a change of lifestyle. There is an ongoing debate on the best strategy for promoting smoking cession, and the effectiveness of confronting smokers with the result of clinical tests such as spirometry. A depressing fact in respect to this was noticed already in 1971 by Cochrane et. al [11]:

> "Probably the best screening test for chronic bronchitis is the question, 'Do you smoke cigarettes?' But unfortunately the subsequent advice is not acceptable."

This illustrates an important point. Most people are aware that smoking is unhealthy, but it can be hard to motivate smoking cession by mere advice. It has however been shown that the ability to give an early COPD diagnosis is an important factor that significantly increases the success rate of smoking cession and life style changes [12, 13], especially at early stages of the disease when the symptoms are relatively mild. An early diagnosis is likewise important for never-smokers exposed to airborne pollutants in other settings such as in the work place. Therefore, there is good reason to believe that an efficient and specific diagnostic technique for detection of the early stages of COPD could be of great value, both for individuals and society as a whole.

# Objectives

The main objective of this thesis is to investigate if measurements of inhaled airborne nanoparticles can be used to obtain clinically relevant information about the human lungs. More specifically this thesis describes a method, referred to as Airspace Dimension Assessment with nanoparticles (AiDA), which was developed and characterised in order to be used for detection of respiratory disease in the distal lung. Specific aims were:

### 1: Development of AiDA theory

To investigate the relation between deposition of nanoparticles in the human lung and lung anatomy and physiology. This includes development of a theoretical model to analyse and understand the deposition of nanoparticles in the respiratory tract during a controlled breath hold. The main purpose of the model is to translate measurements of lung deposition of nanoparticles into clinically relevant variables reflecting lung properties and/or function, in particular the relation between airspace dimensions and particle deposition.

### 2: Development of AiDA instrumentation

To develop an instrument that is able to measure lung deposition of inhaled nanoparticles in a controlled way. This includes optimization of a methodology for generation of test aerosol containing monodisperse nanoparticles, construction of an inhalation system for control of airflows and sampling and development of procedures for measurements of inhaled and exhaled particles.

### 3: Evaluation of the AiDA method in health and disease

To evaluate the AiDA technique as a tool to obtain new information about the lungs. This includes clinical studies characterizing the technique on healthy subjects and subjects with respiratory disease. AiDA measurements were related to and compared with subject characteristics and lung diagnostic techniques.

# Ethical considerations

Research ethics includes the conduct of the individual scientist in relation to methods, honest and transparent reporting of scientific data, independence from conflicting interests, sharing of scientific results, sufficient validation of results and proper crediting of sources of information, data and ideas. It also includes moral obligations to the surrounding society and responsible use of granted resources. Particularly in research including the participation of human or animal subjects, possible ethical complications must be identified and managed.

Besides the compliance to norms of general scientific conduct, the ethical considerations during the work with this thesis mainly concern the balance between the rights and safety of participating subjects and the potential benefits from the scientific accomplishments.

The potential benefits from the research are not hard to motivate. The aim is to develop a technique to detect and diagnose COPD at an early stage. COPD is a common and severe disease that affects a relatively large part of the general population both nationally and worldwide. Current diagnostic techniques are limited. An early diagnosis is critical to the outcome for patients. Thus, an effective method for diagnosis could greatly benefit both individuals at risk for developing the disease and the society as a whole.

If it is found that a diagnostic application is not possible, the minimum scientific output is more general knowledge about the mechanisms governing nanoparticle deposition in the lungs. This is also a scientific field of relevance for human health where the current available data and knowledge are limited.

The potential benefits must however be weighed against the rights and safety of the participating subjects. All studies performed in the scope of this thesis were approved by the regional ethical review board and all studies were performed in accordance to the Declaration of Helsinki including obtaining informed written consent from all research subjects. In addition to information given to the subjects in writing before participation, information was also given orally before the measurements and opportunity to ask questions about the participation was offered. It was also specifically clarified that participation was voluntary and that the subject was free to end participation at any stage of the process. Care was also taken to handle all measurement data in a responsible way and in accordance with applicable rules, including anonymization of measurement data at an early stage in the analysis process.

The AiDA technique is designed to be mild and without adverse health effects. The subject breathes particle free air during most of the procedure. The exposure to nanoparticles during the inhalation phase is at levels comparable to, or lower than, normal urban air, and the used particles are chosen to be without any known toxicity or other adverse health effects. During a typical measurement consisting of a total of 6 breathing manoeuvres it is

estimated that the maximum total mass of particles delivered to the lung is <0.03 µg [14]. This can be compared to the amount of tar delivered to the lung by smoking a normal cigarette which is approximately 30 mg [15] (i.e. 1000 000 times more delivered mass). The type of particles used in AiDA, polystyrene latex nanospheres, have also previously been used in other human studies to investigate mucociliary clearance rates [16-20] and to study particle translocation [21]. There are no known or foreseeable health risks associated with AiDA measurements and we consider the research ethically justified.

# Background

The basis for the technique explored in this thesis is the study of deposition of airborne nanoparticles in the human respiratory tract. Aerosol particle deposition in the lung is a complex process governed by several mechanisms that are dependent on factors such as individual lung geometry, flow characteristics and aerosol particle properties. This chapter provides a theoretical background for some aspects of the involved processes. The chapter includes a brief description of the human respiratory tract, a description of gas and aerosol transport processes in the lung, the most important mechanisms governing lung deposition and their relative relevance in different situations. The chapter also includes a brief summary of previous literature on lung deposition studies, with a focus on experimental studies of nanoparticle deposition in human subjects and aerosol-based diagnostic techniques for the lungs, highlighting some works of special relevance for the work presented in this thesis.

## The structure of the human respiratory tract

The human respiratory tract can roughly be divided into three anatomical regions: the extrathoracic (head) airways, the tracheobronchial zone (conducting airways) and the alveolar (respiratory) zone.

The extrathoracic airways includes the nose, mouth, pharynx and larynx. In this zone the inhaled air is conditioned by warming it to body temperature and increasing the relative humidity. Most inhaled coarse particles (> 10 μm) are also removed in this region by inertial impaction, especially in the region before the larynx where the flowrate is high and the airflow makes a sharp turn. Breathing through the nose provides a more efficient conditioning than mouth breathing, as the surface area is increased and the geometry introduces more turbulence. Hairs in the vestibules behind the nostrils act as an additional protection against inhalation of larger particles.

The trancheobronchial zone includes the airways from the trachea to the terminal bronchioles. The structure of the trancheobronchial zone is often described as that of an inverted tree, with a single trunk, formed by the trachea, and then dividing into smaller and increasingly numerous branches. It should be noted that some individual variation in

branching pattern for some populations have been described [22]. The trachea is normally about a decimetre long and has an average diameter of 18 mm, before it divides into the two main bronchi at the carnia. The main bronchi divide into five secondary bronchi, which then bifurcate for about 15 more airway generations to the terminal bronchioles with a diameter of approximately 1 mm (Table 1, p 36). The conducting airways are surrounded by muscles that can contract or dilate the airways during breathing. The airways in the conducting zone are lined with ciliated epithelium and mucus producing goblet cells, which form the mucociliary escalator that continuously transport mucus upward towards the throat, where it is ingested or removed by chough. Mucociliary clearance is the main removal mechanism for particles that deposit in the conducting airways.

Beyond the terminal bronchioles (around airway generation 17) alveoli start to appear, and the conducting airways gradually transitions into the respiratory zone, where the gas exchange occurs. Alveoli are tiny hollow airspaces that can be found at all airway generations in the respiratory zone. The region of a lung distal to a terminal bronchiole and supplied by a first order respiratory bronchiole is often referred to as pulmonary acini. The respiratory zone includes respiratory bronchioles and alveolar ducts, which terminates in alveolar sacs, normally corresponding to airway generation 23-25. As the main function of this region of the lung is to facilitate close contact between alveolar gas and blood, there is no ciliated epithelium or muscles lining the airspaces in this zone. The alveoli are built up by large, flat type I alveolar cells which form extremely thin and elastic alveolar walls, lined with alveolar lining fluid. A second cell type (type II alveolar cells) produces lung surfactant, which reduces the surface tension of the alveolar lining fluid, and prevents the alveoli from collapsing. The lung surfactant also has an important role in equilibrating the Laplace pressure in differently sized alveoli, enabling uniform ventilation. The alveolar walls also contain macrophages, which are part of the lungs defence system. An overview of the human respiratory system and typical dimensions are given in Figure 2 and Table 1.

It is estimated that there are approximately 480 million alveoli in an average lung [23], and together they make up most of the total lung volume and has a total surface area of approximately 70-100 $m^2$ [24, 25]. The alveoli are surrounded by a web of more than 2000 km of fine capillaries [26], which are perfused by approximately 5 L blood each minute during rest [27]. The blood is equilibrated with the alveolar gas by diffusion of oxygen and carbon dioxide through the extremely thin barrier of the alveoli and capillary walls.

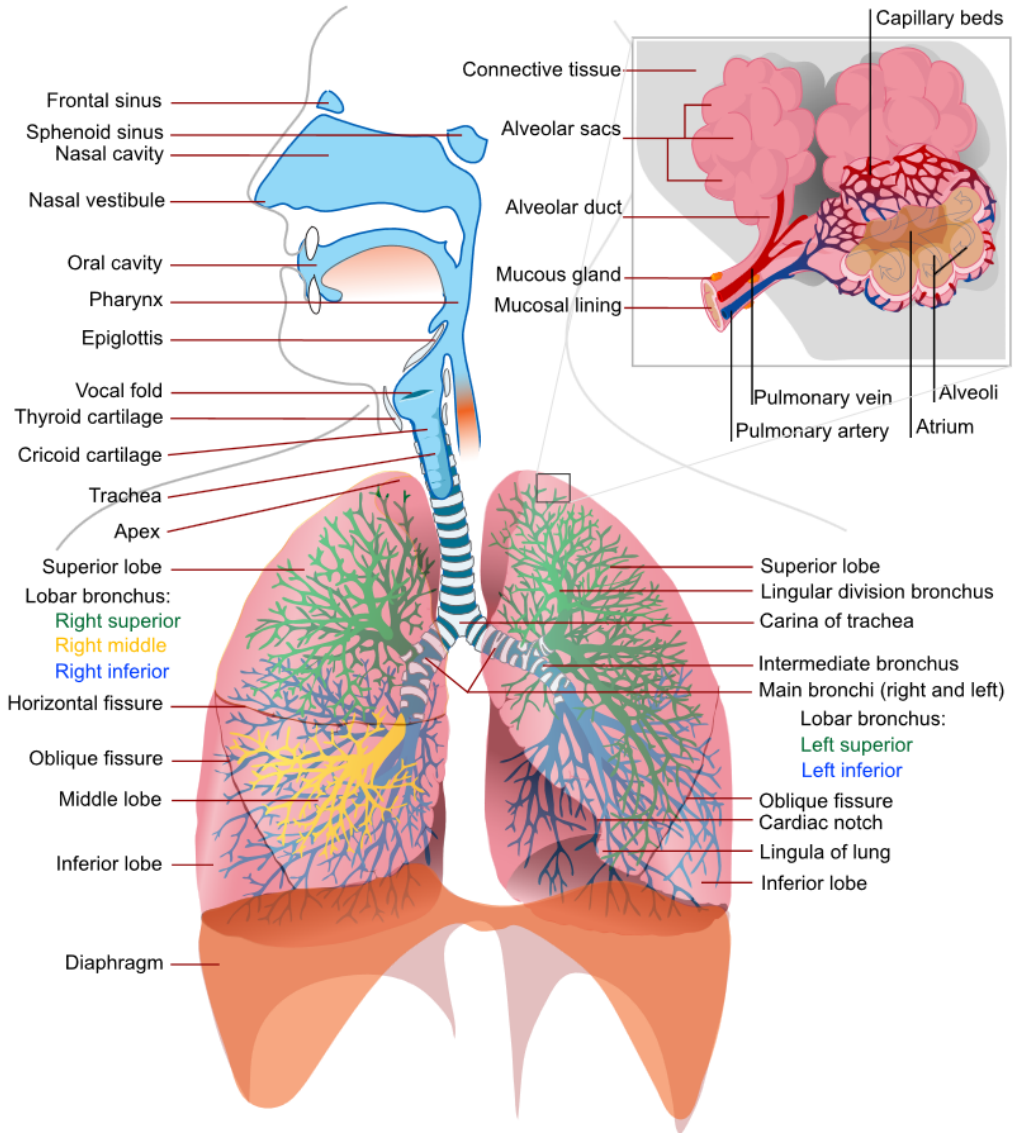


**Figure 2:**
The human respiratory tract. Public domain, creator: Mariana Ruiz Villarreal 2008.

**Table 1:**
Airway generations and typical dimensions in the human respiratory tract, according to the Weibel lung model [19].

| | AIRSPACE GENERATION | | DIAMETER (mm) | LENGTH (mm) | NUMBER | TOTAL CROSS-SECTIONAL ARE, $CM^2$ |
|---|---|---|---|---|---|---|
| CONDUCTING ZONE | TRACHEA | 0 | 18 | 120 | 1 | 2.54 |
| | BRONCHI | 1 | 11.2 | 48 | 2 | 2.33 |
| | | 2 | 8.3 | 19 | 4 | 2.13 |
| | | 3 | 5.6 | 8 | 8 | 2.0 |
| | BRONCHIOLES | 4 | 4.5 | 13 | 16 | 2.48 |
| | | 5 | 3.5 | 10.7 | 32 | 3.11 |
| | TERMINAL BRONCHI | 16 | 0.6 | 1.7 | 60 000 | 180 |
| TRANSITIONAL AND RESPIRATORY ZONE | RESPIRATORY BRONCHI | 17 | | | | |
| | | 18 | | | | |
| | | 19 | 0.5 | 1.0 | 500 000 | 1000 |
| | ALVEOLAR DUCTS | 20 | | | | |
| | | 21 | | | | |
| | | 22 | | | | |
| | ALVEOLAR SACS | 23 | 0.4 | 0.5 | $8*10^6$ | 10 000 |

# Gas and aerosol transport processes in the lung

There are two main transport mechanisms for gas in the lung: convection and diffusion [28]. Both processes contribute to the exchange and mixing of alveolar gas with the inhaled air. Inhaled air is transported by convection through the conducting airways to the respiratory zone of the lung, where mainly diffusion is responsible for the gas exchange between the inhaled air and alveolar gas. The same transport processes are also responsible for transport of inhaled aerosol particles [2], but it is important to make a distinction between the theoretical treatment of gas and particles, as their physical properties differ. For gas, convective transport is considered reversible and diffusion irreversible, while for aerosol particles both convective and diffusional transport can be considered irreversible. In addition, particles move by gravitational settling and inertia [26].

Convective transport is the bulk transport of gas caused by hydrodynamic pressure gradients. This transport process moves all individual components in the inhaled gas/aerosol regardless of individual concentrations in the mixture. In the lung, convective transport during breathing is facilitated by the action of the diaphragm and inspiratory

intercostal muscles. During inhalation the action of these muscles enlarges the thorax, which lowers the pulmonary pressure and thus causes air to flow into the lung. This is followed by relaxation of the muscles, which allows the chest walls and lungs to passively return to their original dimensions and temporarily compress the air, which therefore flows out of the lung during exhalation. Thus, in a healthy lung, inspiration is active and exhalation is passive. During convective transport, the flow can be laminar, turbulent or mixed. In the lung, the flow is normally only turbulent in in some parts of the large central airways where the linear gas velocity is high [29], and laminar in the smaller airspaces where the combined cross sectional area is high and the linear gas velocity is low. Convective flow is most commonly described by ideal flow fields, representing the linear gas velocity in the flow. In these representations, convective transport of gas molecules follows the flow field accurately and reversible. Compared to gas molecules the behaviour of aerosol particles in convective flow is more complex, as the particles have more mass and inertial momentum and, depending on particle properties (such as size, density and shape) will follow the ideal flow field to a variable degree. That convective transport of aerosol in the lung is irreversible has been clearly shown by bolus dispersion experiments [30].

Diffusion is the net transport of molecules or particles in concentration gradients caused by Brownian motion. The transport direction is always opposite to the concentration gradient (toward lower concentrations). In healthy lungs, diffusion is the main mechanism for gas exchange between the inhaled air and alveolar gas in the respiratory zone. As oxygen is continuously absorbed and transported from the alveolar gas to the blood, there will always be a concentration gradient present driving diffusive transport of oxygen from the inhaled air to the alveolar gas. In the same way, carbon dioxide is desorbed from the blood to the alveolar gas and diffusively transported to the tidal air which is then exhaled. Because of the rapid diffusion rates of gas molecules, the tidal air and the alveolar gas will approximately reach a well-mixed steady state during slow normal breathing [31].

Diffusion is also an important transport process for aerosol particles in the lungs. It was shown by Einstein in 1905 [2] that gas molecules and aerosol particles are affected in the same way by Brownian motion and diffusion, and that aerosol particles conceptually can be viewed as enormous and very slow gas molecules. As diffusion is strongly depending on particle size, it is most important for particles < 100 nm, and limited for particles > 1 μm in the lung. If an aerosol containing a high concentration of nanoparticles is inhaled, diffusional transport toward the generally low particle concentrations in the alveolar gas will occur. In addition, aerosol particles that reach a surface in the lung deposits and are thus removed from the aerosol. Therefore, all surfaces in the lung will act as particle sinks which cause the formation of concentration gradients and diffusional transport toward surfaces. This will be further discussed in the following section about particle deposition mechanisms. The diffusion rate of particles is, however, orders of magnitude slower than for gas molecules, and in contrast to gas molecules, the extent to which an inhaled aerosol mixes and reach equilibrium with alveolar gas is not entirely clear. Therefore, it is important

to recognise that even though gas molecules and aerosol particles in principle are transported by the same processes in the lung, they will behave differently from a practical point of view.

## Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease is defined as a chronic airflow obstruction that is not normalized after bronchodilation, combined with prolonged productive cough, dyspnoea at exertion and history of exposure to tobacco smoke or other hazardous agents [32, 33]. More precisely COPD is an umbrella term, which refers to a group of diseases composed of mainly two components: changes to the conducting airways (bronchitis/bronchiolitis) and emphysema [34]. The conditions can be present at various degrees in different phenotypes, which complicates the diagnosis of the disease.

Changes to the terminal and transitional bronchioles are generally considered to commonly be the first pathological changes to the lungs in COPD [35]. It has been shown that even at moderate stages of COPD the changes can cause remodelling of the small airways [36], with a reduced number of terminal and transitional bronchioles and the remaining small airways having thickened walls and narrowed lumens. The remodelling process in the small airways is closely related to the decline in lung function in patients with COPD [37].

Emphysema is defined as enlargement of airspaces distal to the terminal bronchioles, with destruction of alveolar walls. Emphysema can be centriacinar (affecting the acinus but not the most distal alveoli) or panacinar (affecting all distal airspaces), and distributed more or less evenly over the lung. Extensive emphysema damage can result in large air pockets or voids in the lung called bullae. Emphysema results in loss of lung surface area and lung elasticity [34], which causes obstruction and impaired gas exchange between alveolar gas and the pulmonary circulation.

Early symptoms of COPD are often experienced as repeated “colds” with cough for extended periods of time. Other early symptoms include wheezing or whistling sounds from the chest. These symptoms usually occur at an early stage and do not correlate with the severity of the disease [38, 39]. As the disease progress the most disabling symptom is gradually increasing dyspnoea, which increases with the severity of the disease.

Smoking is generally regarded as the largest contributor to the COPD burden in parts of the world with a high socio-demographic index (SDI) while environmental exposure is believed to have a higher influence in countries with lower SDI [5]. As tobacco use decreases, environmental and occupational exposure are increasingly important risk factor for COPD [10].

# Particle deposition in the human lung

When aerosol particles come in contact with a surface they generally attach firmly to it by van der Waals forces, electrostatic forces or by forces that arise from the surface tensions of liquid films [26]. Normally they do not detach after deposition.

Particle deposition in the lung is difficult to predict by theoretical aerosol models, as the processes occur in a system of continuously changing geometry and flowrates, which varies over time with the breathing cycle. A precise analytical description of aerosol dynamics in a human lung would require a complete modelling of the respiratory airflows in the lung followed by superimposing particle motions on this solution. To accurately determine particle deposition in the lung would require determination of boundary conditions describing the changing lung geometry and flows followed by integration of the aerosol dynamics over time [26]. To date this is not possible, but several semi-empirical models for predicting particle deposition have been developed [40-42], and an analysis of the involved mechanisms can provide some understanding of factors that determine lung deposition, and their relative impact. Lung deposition fraction as a function of particle size for different regions of the lung and total deposition fraction estimated with the International Commission on Radiological Protection (ICRP) model [40] is shown in Figure 3.

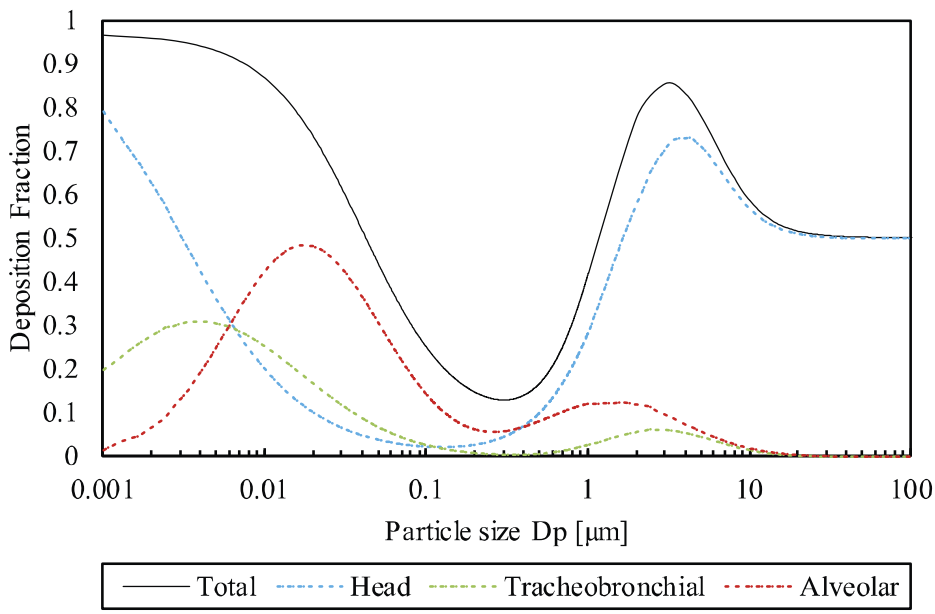


**Figure 3:**
Particle deposition fraction as a function of particle size in different regions of the respiratory tract, estimated by the ICRP model for a healthy adult male during light exercise [38].

The total lung deposition is a sum of several superimposed deposition mechanisms with different relevance for different particles, flow conditions and regions of the lung (Figure 4). During convective transport in larger airways the most important deposition mechanism for nanoparticles is convective or eddy diffusion, where small aerosol "parcels", because of turbulence or eddies in the airflow, are transported close to surfaces and particles deposit. Turbulent diffusion is dependent on the airflow geometry and complicated to describe in detail for lung geometry.

The dominant deposition mechanisms in the more peripheral lung, where distances are shorter and flow rates lower, are impaction, sedimentation and diffusion. In special situations interception and electrostatic deposition can also be relevant deposition processes.

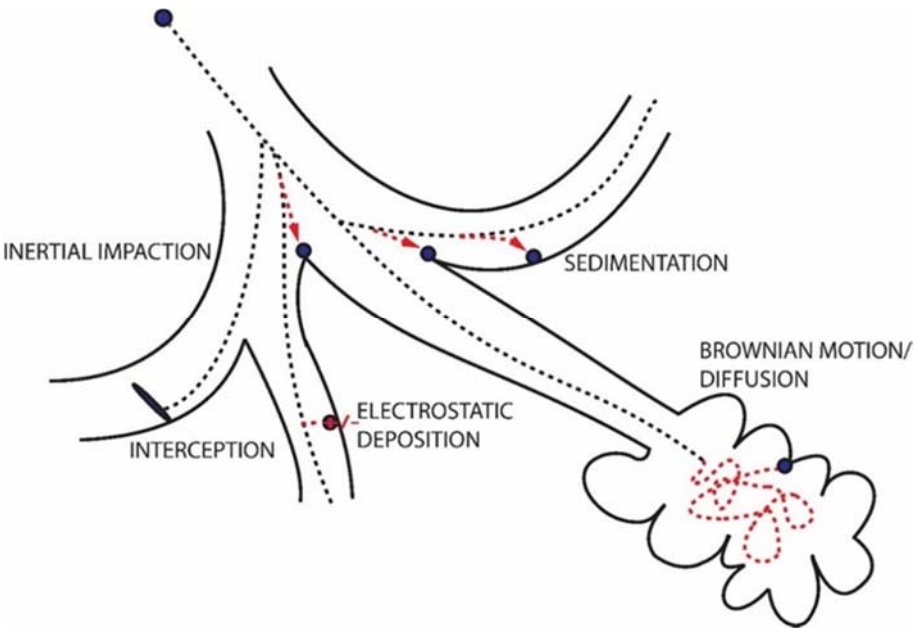


**Figure 4:**
The dominant deposition mechanisms in the distal lung are impaction, sedimentation and diffusion. In some situations, also interception and electrostatic deposition can be relevant mechanisms.

## Impaction

Inertial impaction occurs when a particle is moving in a bulk gas flow that encounters an obstacle and changes direction. If the particle has enough inertial momentum, it will not be able to adjust quickly enough to the change in direction of the flow and thus crashes into the obstacle. Impaction can be described by analysis of the Stokes number (Stk) which is a dimensionless number defined as the ratio of the moving particles' stopping distance (*S*) and a characteristic dimension of the obstacle.

An analysis of the factors determining the stopping distances shows that impaction is most important for dense particles at high flow rates, especially at sites where the respiratory flow changes direction, such as bifurcating airways or at changes in airway geometry where the gas is suddenly accelerated or deaccelerated.

## Sedimentation

Gravitational sedimentation or settling is the deposition of particles by gravitational forces. The most important variable for describing sedimentation is the terminal settling velocity, $V_t$, which is the constant velocity of a falling particle in dynamic equilibrium. Sedimentation is most important for particles with high density and mass in long, horizontal airways, where the respiratory flow rate is low and the residence time thus relatively high.

## Diffusion

Aerosol particles are affected by Brownian motion caused by collisions between the particle, gas molecules and other particles. If there is a concentration gradient present statistically more collisions will occur on the side with the highest concentration of other particles, and this results in a net transport of material, namely diffusion. Particles that reach a surface deposit and thus are removed from the bulk of the aerosol. This causes a concentration gradient to evolve over time toward all surfaces, and diffusional transport and deposition occur.

The rate of diffusion is dependent on particle size, but not on density [26]. The characteristic variable describing diffusion is the diffusion coefficient, $D$. Diffusion is the dominant deposition mechanism for particles < 100 nm in the lungs and limited for particles larger than 1 µm. Most diffusional deposition occurs in the alveolar zone where distances are short and flow rates are low, with exception for the smallest particles (< 20 nm) which to a large extent deposit in the head and upper airways because of their rapid diffusion rate [40].

The relative importance for inertial impaction, sedimentation and diffusion are shown in Figure 5:

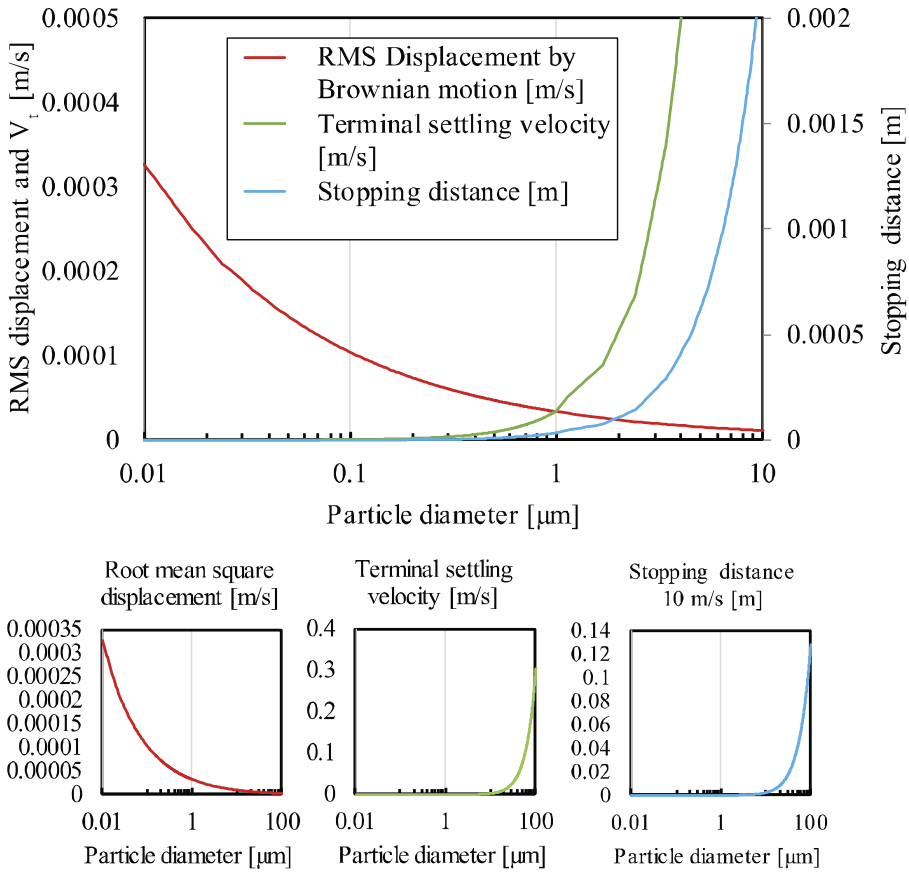


**Figure 5:**
The relative importance of deposition mechanisms for particles in the size interval 10 nm - 10 µm, represented by the terminal settling velocity for sedimentation, the root mean square displacement by Brownian motion for diffusion and the stopping distance for a particle travelling at 10 m/s (relevant velocity for inhalation) for impaction. The stopping distance values are shown on the secondary (right) axis. Values for the size range 10 nm – 100 µm for the different mechanisms are shown in the three lower panels. The figure shows that for particle sizes relevant for the AiDA method, (< 100 nm) diffusion is dominant [26].

## Interception

Interception occurs when a particle is moving in a bulk flow that passes close to a surface so that the surface come within a distance corresponding to one equivalent particle radius and thus the particle comes into contact with the surface and deposits. The difference between interception and impaction is that in the former case, the particle deposits without deviating from the bulk flow. Interception is most important for particles with a high aspect ratio, such as fibrous materials.

### Electrostatic deposition

Highly charged particles may deposit by electrostatic deposition, caused by the electrostatic image charge they induce in the airway surface. Unipolarly charged particles with high concentrations may also deposit because they are driven toward the lung surface by mutual electrostatic repulsion. However, under normal conditions this is not usually considered a relevant mechanism as the lung surface can be regarded as conducting.

## Particle deposition in the diseased lung

Most knowledge about particle deposition in the lung is limited to models of the healthy lung. To date, only few studies provide experimental data for lung deposition of nanoparticles in diseased lungs, including studies performed in the work related to this thesis [14, 43-48]. More data is available for larger particles [30, 49-52]. Efforts have also been made to model particle deposition in diseased lungs [53-57] but as discussed previously, accurate modelling of particle deposition even in normal lungs is challenging and alterations in diseased lungs can take many forms, which increases the complexity of the situation further. Therefore, experimental data is essential for understanding particle deposition in diseased lungs, and collection of more reliable data of high importance.

Alterations to lung function and structure can be assumed to cause more or less significant changes for the deposition efficiency of inhaled particles, depending on the disease and the properties of the inhaled particles. Generally, changes to the conducting airways, such as bronchitis/bronchiolitis and altered bronchiole branching patterns [22] or remodelling [36] can be assumed to increase particle deposition, especially for larger particles susceptible for impaction. In contrast, enlarged airspaces can have the opposite effect, especially for nanoparticles. It can also be noted that the actual inhaled dose of airborne pollutants is not just depending on deposition efficiency, but also minute ventilation. This is discussed in Paper I and III, and understanding of the effects of altered lung morphology on lung deposition is the basis of the AiDA technique.

## Review of previous literature of lung deposition

The first scientific description of lung deposition experiments are probably the observations of John Tyndall (1820-1893) in his "Essays on the floating-matter of the air in relation to putrefaction and infection", where experiments are described that shows that the air which has passed the lungs is depleted from particles scattering light [58]. The immediate

relevance of his discovery does not appear to have been evident for Tyndall other than on a philosophical level as he wrote:

> "What is the practical use of these curiosities? If we exclude the interest attached to these observations of new facts, and the enhancement of that interest through the knowledge that facts often become the exponents of laws, these curiosities are in themselves worth little. They will not enable us to add to our stock of food, or drink, or clothes or jewellery. But though thus shorn of all usefulness in themselves, they may by carrying thought into places which it would not otherwise have entered, become the antecedents of practical consequences."

Since the observations of Tyndall the scientific body of knowledge has increased considerably. Scientific work on lung deposition has however until recently focused mostly on the deposition of micrometre-sized particles. This emphasis on larger particles is likely both due to limited instrumentation and, until recently, a limited understanding of the importance of nano-sized particles for human health.

There are several reasons to measure lung deposition of inhaled particles, including, but not limited to, dose assessment of inhaled medicine, estimation of exposure to airborne pollutants and measurements of lung properties.

For the sake of compendiousness, the focus of this brief review will be limited to studies featuring lung deposition measurements of nanoparticles and lung deposition studies with the aim to derive information about properties of the lungs.

## Lung deposition of nanoparticles

Studies describing lung deposition of nanoparticles (< 100 nm) are scarce, and to date only about 50 publications describe experimental determination of lung deposition of nanoparticles [59]. In total, excluding the subjects participating in our studies, data for somewhere between 500 – 600 research subjects have been reported in total. The number of subjects included in the studies is typically low (n < 10), with some exception where data for up to 36 subjects have been included [60].

Several different aerosol sources have been used in the studies, ranging from ambient air [61], to diesel exhaust [62], tobacco smoke [63], or aerosols produced by spark discharge [46] or evaporation/condensation aerosol generators [64]. Several different breathing patterns have been used and different measurement techniques have been applied ranging from filter collection [65] and impactors [66] to scanning mobility particle spectrometer systems (SMPS) [67] and gamma cameras [68]. The scarcity of data and diversity of methods result in large uncertainties regarding absolute deposition efficiency for aerosol particles < 300 nm.

## Aerosol based diagnostic techniques

Several different approaches to inhalations of aerosol particles have been used to assess features of the lungs, including inhalation of radioactive tracer particles, which can be used to visualize deposition efficiency and the sites of deposition by medical imaging techniques [69, 70], and studies measuring the uptake and clearance of inhaled substances [71]. This section will be focused on techniques that use direct measurements of aerosol particles to derive information about the lungs.

Early experiments with the aim to use lung deposition measurements directly for respiratory disease were performed in the 1960s where a difference in aerosol recovery was observed between normal subjects and patients with respiratory disease [72-75]. From these initial results several different techniques have been developed and refined. The methods can generally be divided in two main categories based on the way the test aerosol is administered to the subject: Aerosol Bolus Dispersion techniques and Aerosol-Derived Airway Morphometry (ADAM).

Aerosol Bolus Dispersion techniques aim at examining convective gas mixing in the lungs. In short, the technique is performed by inhaling narrow boluses of test aerosol at constant flow rate to a desired volumetric lung depth and then exhalation at the same constant flow rate. The particle concentration is measured at inhalation and exhalation by a light scattering device, and several parameters such as the broadening of the bolus peak, the recovery of particles, the modal shift and the skewness of the bolus peak are determined. From these parameters data about the mixing in the respiratory system can be inferred. The test aerosol generally contains monodisperse droplets of hydrophobic substances in the size range 0.4-1 μm, such as di(2-ethylhexyl) sebacate (DEHS). The test aerosol is chosen so that the particles should have minimal diffusive motion and a well-defined gravitational settling velocity [30].

Studies have shown that the technique has some sensitivity for asthma for patients with abnormal pulmonary function tests [76], but not for asthmatics with normal tests [77]. The techniques have also shown the ability to detect changes caused by broncho-provocation with methacholine [78-81], cystic fibrosis [82], obstructive disease [83] and differences between smokers and non-smoking subjects [84-86].

In a comprehensive review by Blanchard it is concluded that aerosol bolus techniques are simple to administer and capable of detecting known alterations to the lung [30]. However, it is also concluded that every condition detected by the techniques is also detected with other conventional, more established lung function tests [30].

Aerosol-Derived Airway Morphometry (ADAM) is a group of techniques aimed at assessing airway and acinar diameters, similar to the technique proposed in this thesis, but with larger particles (typically 0.8 -1 μm), assumed to deposit solely by sedimentation. Theoretical

models have been derived for interpreting the measured recovery of particles to airway dimensions [87, 88]. The results from the technique can be expressed in an Effective Airway Diameter (EAD), which can be measured as a function of volumetric lung depth. Three versions of the technique have been described: the single-breath recovery technique, the bolus recovery technique and the particle concentration technique [49, 89].

The single-breath recovery technique closely resembles the technique proposed in Paper II in this thesis in practical execution and the theoretical determination of airspace dimensions proposed in Paper I. A large volume of test aerosol is inhaled and the particle recovery is determined for several breath-holding times. By determining the half-life time of particles in the lungs one value for the volume-weighted average EAD can be determined for the whole lung. As the major part of the lung volume resides in the acinar region of the lungs, the EAD derived from this version of ADAM is weighted toward the dimensions in this part of the lungs [90].

The Bolus Recovery technique uses narrow boluses of test aerosol (30-100 mL) inhaled to predetermined volumetric lung depths to probe the dimensions of specific parts of the lungs, thus providing greater volumetric resolution of the lungs and regional data. To chart the lungs, boluses to several different volumetric lung depths, and several breath-holding times are required. This provides EADs as function of volumetric lung depth (absolute or relative). The measurements required to chart the lungs in this way can be time consuming. Typically five different lung depths and six breath-holding times on each lung depth (= 30 measurements) have to be performed to make the lung assessment [91]. This version of ADAM is very similar to the method described in Paper IV, but instead of filling the lung with test aerosol and analysing a small sample from a determined volumetric sample depth, a small bolus is given during the inhalation and all exhaled air is analysed.

The particle concentration technique resembles the single-breath technique in execution, but the particle concentration in the exhaled breath is measured continuously, and the concentration signal is divided into bins. The recovery from each of these bins, corresponding to a specific volumetric lung depth, is determined relative the neighbouring bins and in this way several volumetric lung depths can be probed simultaneously. By performing the manoeuvre for a few breath-holding times the half time of aerosol particle concentration can be determined at different volumetric lung depths, and thus the EAD for these lung depths [89].

The validity of the different versions of the ADAM technique has been evaluated by different means in several studies with convincing results. Several studies have compared the EADs derived from ADAM to established lung models [90, 92-94]. The theoretical model for ADAM has been validated by comparing theoretical predictions from the model to experiments with physical models with known dimensions with favourable results [95]. A few studies have been performed by comparing EADs to morphometric measurements in excised human lungs [96], in donkey lungs [97] and in dog lungs [98]. Most of the

studies show reasonable agreement between the results derived from ADAM and the reference methods. The aggregated body of evidence makes a convincing argument that the foundations for the method are sound.

ADAM has also been used in several studies comparing EADs derived from the technique to established lung function indices for healthy and diseased subjects to the effects of medical interventions and efficacy of therapy. The studies have shown that EADs are independent of most anatomical parameters and lung function tests for healthy subjects, but significant correlations between EADs and lung function tests for patients with lung disease have been observed [92-94, 99]. Especially significant is the correlation between EADs and emphysema [100-102]. An important exception for the independence of EADs to anatomical parameters is that of age [94, 103]. This is according to expectations, as airspace dimensions are believed to increase with age.

Compared to the Aerosol Bolus Recovery technique, ADAM has similar advantages. The different versions of the technique are fairly simple to administer and have high reproducibility, specificity and sensitivity to different lung conditions. In addition to these attractive features, it has been suggested that ADAM can provide information not easily accessible by current standard lung function tests [49]. Nevertheless, even though the technique has several apparent advantages, ADAM has not been adapted in clinical practice as a standard technique and the publications on the subject has ceased over the last decade. Reasons for this may be that the technique has to be performed at slow and constant flow rates which can be challenging for some subjects, especially subjects with respiratory disease, and the lack of established reference values for normal subjects.

# Methods

This section briefly describes the methodology used in the studies included in this thesis and related work, with a focus on the AiDA technique. The methodology has evolved over time; therefore, it may not be obvious why somewhat different approaches to the AiDA technique has been applied in the different studies. The aim of this section is to clarify the theoretical basis for the AiDA method, and how it has evolved.

## Study design

Several different approaches were adapted to investigate different aspects of the AiDA technique. As previously reported in the background section, data on experimental lung deposition of nanoparticles is limited and the presented methods diverge, thus the initial expectations on the technique were uncertain. This lead to a gradual development of the methodology.

Importantly, the development of theory, analysis and data collection for the papers in this thesis were to some extent done in parallel, thus the order of publication does not automatically follow the chronological order in which the studies were performed. For instance, this explains why only lung deposition fraction was measured for Paper III and not the data needed for determination of effective airspace radii and zero second recovery. To give an overview of the methodology adapted in the different studies, and the different approaches to the AiDA method, a short summary of the studies is given in the following section. A summary of the approaches to the AiDA technique is also provided in Table 2, on page 52.

### Paper I: The theory

Paper I introduces the theoretical background for the AiDA technique, its expected advantages and limitations and the model for derivation of effective airspace radii, ($r_{AiDA}$) from particle recovery. It was also suggested that the particle recovery after zero seconds, ($R_0$), may be used to infer information about the small conducting airways.

The model is an adaption of a model originally described by Goldberg [104]. The model is compared to a different model that previously has been used for similar purposes with larger particles (ADAM) [87, 90]. The article presents no original experimental data, but summarizes previous findings of studies on nanoparticle deposition in subjects with respiratory disease and examines AiDA from a theoretical perspective. The paper discusses AiDA as a possible diagnostic technique for respiratory disease and suitable experimental conditions and technology for performing measurements on human subjects.

### Paper II: The instrument

Paper II is a method article, describing the AiDA instrument constructed according to the recommendations in Paper I. The instrument is characterized by measurements on a small group of young, healthy subjects (n = 7). Measurements are performed with different particle sizes (22, 50, 75 and 100 nm) and with different breath holding times (3 – 20 s) from a fixed sample depth of 1800 mL. The particle losses in the instrument are determined and a model to account for these losses during measurements on human subjects is presented. The influence of flow rates was examined in the interval 1 – 10 L/s, and the results of measurements on the subjects is compared to data calculated with the multiple path particle dosimetry model (MPPD, v. 2.1) [41].

### Paper III: Measurements on subjects with respiratory disease

Paper III reports measurements on three groups of subjects: healthy never-smokers (n = 17), active or former asymptomatic smokers (n = 15) and subjects with diagnosed COPD (n = 16). The COPD subjects were prospectively recruited and defined by using the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [33]. The control group and the group of asymptomatic smokers consisted of a convenience sample. The groups were matched with regard to age, sex, weight and length. All subjects performed lung deposition measurements with 50 nm particles from 1300 and 1800 mL volumetric sample depth and with 100 nm particles from 1800 mL volumetric sample depth with the AiDA instrument. To assure the quality of data two criteria were set up for acceptable AiDA measurements in this study: an inhalation of > 70 % VC of the test aerosol and a complete breathing manoeuvre in less than 17 seconds, including a 10 s breath hold. All subjects performed pulmonary function tests, and the COPD group also underwent a CT scan of the lungs. The tested hypothesis was if particle deposition measured with the AiDA instrument would show differences between the different groups of subjects, and if the differences could be explained by changes in lung morphology. The CT results are described in more detail in a separate publication not included in this thesis [14].

### Paper IV: $R_0$ and $r_{AiDA}$ on varied sample depths

Paper IV explores $R_0$ and $r_{AiDA}$ on a group of 19 healthy subjects (aged 17-67 yrs.) at different volumetric sample depths from approximately 200 mL to measurements close to full vital capacity. The particle size used was 50 nm for all measurements. Paper IV can be seen as a "map" of recovery measurements with the AiDA instrument, and a basis for designing future AiDA measurement protocols. For each subject 48 measurements of recovery were made. Measurements were performed at five volumetric sample depths with an upper sample front of 700, 1000, 1500, 2000 and 2500 mL and an additional measurement at a volumetric sample depth individually adjusted close to full vital capacity. All subjects also performed pulmonary function tests, including measurements of diffusing capacity for carbon monoxide in the lung, ($D_{L,CO}$).

## Data collection for future publications

Based on the experiences from working with the AiDA technique, an efficient measurement protocol was designed for determination of effective airspace radii ($r_{AiDA}$) and the zero second recovery ($R_0$) in a limited number of breathing manoeuvres. The standard protocol includes a total of 6 measurements of particle recovery with 50 nm particles from a fixed volumetric sample depth at 1300 mL, chosen to represent gas from the respiratory zone of the lung. The measurement protocol can usually be performed in less than 15 minutes for most subjects, and generates one $r_{AiDA}$ value and one $R_0$ value. As criteria for data quality, the goodness of fit, defined by the coefficient of determination, r, was used. A coefficient of determination > 0.95 was considered the criteria for a successful measurement. The measurement protocol has been performed by a large number of subjects (n > 1000). These research subjects were participating in several studies, and some preliminary data and results will be presented and discussed within this thesis.

### The Swedish CArdioPulmonary bioImage Study (SCAPIS)

The Swedish CArdioPulmonary bioImage Study (SCAPIS) [105] was the largest study where AiDA measurements were included. In this study complete and quality-controlled AiDA measurements were accomplished for 671 research subjects. SCAPIS is a major joint national effort in Sweden to reduce mortality and morbidity from cardiovascular disease, COPD and related disorders [105]. The main objectives of the study is to characterize a Swedish cohort of 30 000 subjects aged 50 – 64 years to obtain novel information with the aim to identify and treat individuals with disease, and to optimize the ability to investigate disease mechanisms [105]. SCAPIS is designed as a prospective observational study of a

randomly selected sample from the general population. Subjects are requited and examined at six university hospitals in Sweden, with examination of 5000 subjects at each site [105].

The SCAPIS measurement protocol included detailed questionnaires, collection of bio samples, anthropometry, electrocardiography as well as lung function tests, computed tomography (CT) and magnetic resonance imaging (MRI) [105]. The lung function tests included dynamic spirometry, measurements of forced expiratory volume in 1 s ($FEV_1$), forced vital capacity (FVC) and single-breath diffusing capacity of carbon monoxide ($D_{L,CO}$) according to current recommendations [106, 107]. In addition to the standard SCAPIS protocol, optional examinations were allowed to be included at the sites where the measurements were performed. In Malmö, AiDA measurements were included for 736 subjects.

The data from the SCAPIS study is not yet fully analysed, but a short overview together with some preliminary results are presented in the Results and Discussion section of this thesis. The number of subjects that has participated with AiDA measurements in the SCAPIS study exceeds the total number of subjects in all previously published studies giving data on measurement of lung deposition of nanoparticles. A summary of the approaches to AiDA measurements is provided in Table 2:

**Table 2:**
An overview of different approaches to AiDA in the papers included in this thesis, and the measurements performed under the scope of SCAPIS.

| Paper | Particle size [nm] | Breath hold [s] | Vol. [mL] | Subjects | Reported data |
|---|---|---|---|---|---|
| **I.** | na | na | na | na | na |
| **II.** | 22, 50, 75, 100 | 3, 5, 7,10, 20 (x3) | 1800 | 7 Healthy | *R* |
| **III.** | 50, 100 | 10 (x3) | 1300, 1800 | 17 Healthy<br>15 Smokers<br>16 COPD | *DF* |
| **IV.** | 50 | 5, 6, 7, 8, 9, 10, 15, 20 | 200 - Max | 19 Healthy | $r_{AiDA}$, $R_0$ |
| **SCAPIS** | 50 | 5, 7, 10 (x2) | 1300 | >671 | $r_{AiDA}$, $R_0$ |

# The concepts of AiDA

Aerosol particle deposition is determined by the properties of the particles, the flow conditions and the geometry of the structure in which the aerosol resides. The relation between these factors is determined by aerosol physics. If the properties of the aerosol, the flow conditions and the resulting particle deposition are all known, aerosol physics can be used to infer information about the geometry of the system.

In the AiDA technique this idea is applied to the human lung, and the factors are controlled to simplify the situation. This enables the construction of models which can be used to

infer information about the lung. How the factors are controlled is perhaps most easily explained by a step by step walk-through of the standard measurement procedure (Figure 6).

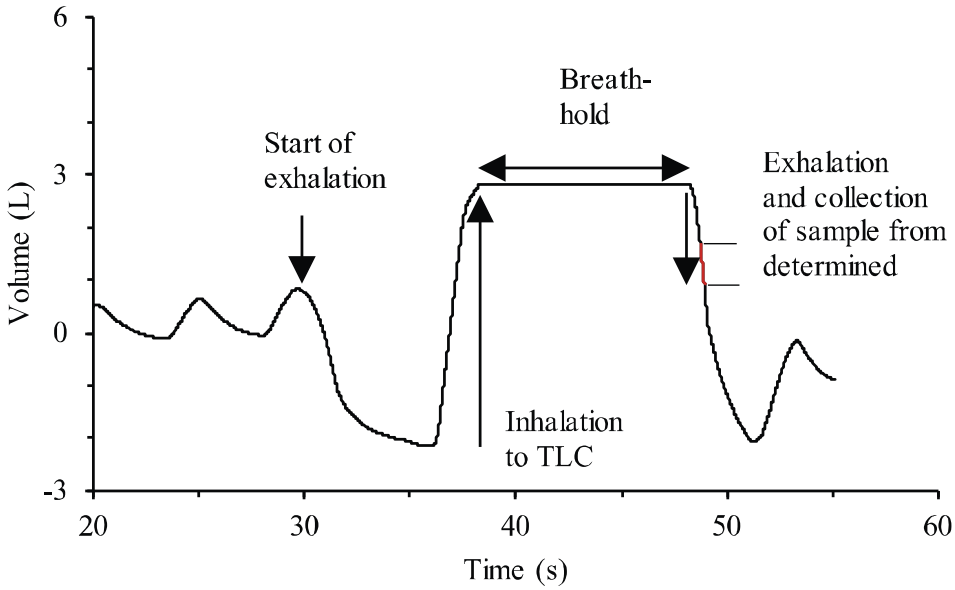


**Figure 6:**
The breathing pattern used for a standard measurement of particle recovery in the AiDA instrument.

The measurement procedure starts with the subject ventilating the lungs by normal tidal breathing of particle free air through a mouthpiece. During this period the lungs are cleared from any ambient particles residing in the lung.

The ventilation period ends with an exhalation to residual volume (RV) followed by an inhalation of monodisperse test aerosol with known particle concentration to total lung capacity (TLC). By using a test aerosol containing monodisperse, spherical nanoparticles of known size, the analytical description of the aerosol can be greatly simplified. The nanoparticles will readily follow the convective air movements with minimal (but not negligible) influence of flowrate dependent processes such as inertial impaction and turbulent diffusion. This enables the inhaled nanoparticles to efficiently reach the convection/diffusion front in the respiratory zone of the lung where the exchange between inhaled air and alveolar gas occurs mainly by diffusion.

When the subject has reached full inhalation to TLC, a respiratory pause follows. During this period, practically no convective air movements affect the inhaled aerosol and the behaviour of the inhaled nanoparticles will be controlled almost exclusively by diffusion. The simplification that no convective movements occur during the breath hold is justified

by low flowrates in the distal lung. As the lung is ventilated with particle free air before the inhalation the direction of the diffusional transport is in the direction toward lower concentration, toward all surfaces and toward the particle free alveolar gas volume. This is an important detail because it means that if the diffusional particle deposition is thought of as a "ruler" to measure distances in the lung structure, the direction of the measurement is toward all surfaces and residual airspaces simultaneously.

After a determined breath hold time the subject exhales, and the nanoparticles that have not deposited or moved into the residual volume follow the exhaled tidal air by convection. A sample is collected from a controlled volumetric sample depth of the exhaled air and analysed. The particle concentration in the exhaled sample is compared to the reference inhaled particle concentration and reported as particle recovery, ($R$), which is the fraction of particles that are recovered in the exhaled sample, or as the deposition fraction ($DF$) which is the fraction of the particles which have been removed from the inhaled aerosol during the breathing manoeuvre. By convention particle recovery, $R$, will be used when discussing the AiDA technique, as the particle concentration in the exhaled air is the primary data collected by the instrument.

## Analysis of AiDA measurements

By adapting the above described measurement procedure, two distinct phases of the measurement can be identified: 1) A dynamic phase when the test aerosol flows through the conducting airways, and 2) a static phase, when the aerosol resides in approximately still air in the alveolar region of the lung. The measured particle recovery is a function of the processes during both phases. This presents a problem, as different processes are acting during the two phases, and a single measurement of recovery is unable to distinguish between the processes and only gives information about total deposition, as in Paper III.

By performing a series of recovery measurements at different breath holding times more specific information can be obtained. This approach enables a distinction between particle recovery during the two phases of the breathing manoeuvre by determining the relation between particle recovery and residence time in the lung by an exponential curve fit, as seen in Figure 7.

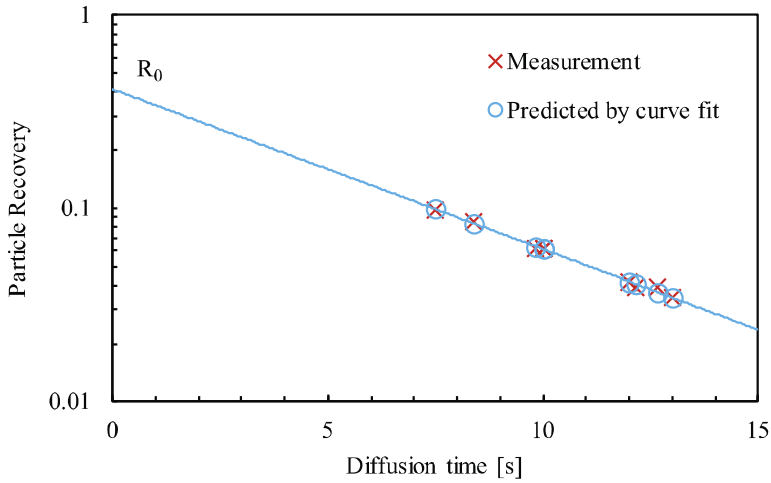


**Figure 7:**
Data analysis of a standard AiDA measurement at a fixed volumetric sample depth (1300 mL), 50 nm particles and duplicate measurements at 5, 7 and 10 s breath holds. Natural variations in the subjects breathing patterns causes a spread of aerosol residence time in the lung.

The particle losses that occur during the dynamic part of the breathing manoeuvre (i.e. inhalation and exhalation, which are approximately equal irrespective of breath holding time) can be estimated as the y-axis intercept of the fitted recovery decay curve at t = 0 s. This is termed the zero second recovery, $R_0$. The diffusional deposition rate during the breath hold can be determined from the exponent of the decay curve and reported as the particle half-life time in the lung ($t_{1/2}$). As we will see, this variable can be used to estimate effective airspace dimensions.

## The Zero Second Recovery, $R_0$

The zero second recovery, $R_0$, can conceptually be understood as an estimation of particle recovery after a breathing manoeuvre without a breath hold. This should correspond to particle losses during the dynamic part of the breathing manoeuvre, when the aerosol flows through the conducting airways. Even though the theoretical interpretation of $R_0$ is appealing, the real situation is somewhat more complicated, as the respiratory flow of aerosol is not fully reversible. The particle losses are most likely to be dominated by diffusion losses during transport, but it is also possible that effects such as convective mixing with residual air or air trapping in the lung due to airway closure can be influential factors. The current understanding of $R_0$ is based on the findings in Paper I, III and IV included in this thesis, and preliminary results included in the result section (Table 6) and a related

publication [108]. It is likely that $R_0$ may reflect properties of mainly the small conducting airways. To date there is no comprehensive theoretical model to interpret $R_0$, especially in subjects with respiratory disease, and the true nature of this variable remains to be studied in future work.

## The Effective Airspace Radius, $r_{\mathrm{AiDA}}$

The main original objective of the AiDA technique was to assess the dimensions of distal airspaces of the lung and thus it is necessary to establish a model that describes the relation between particle recovery during the breath hold and the geometry of the lung.

The following section will outline the derivation of the model, but not provide the derivation in its entirety, as this is described in the original paper [104]. As we shall see it arrives at a surprisingly simple relation between the particle half-life time in the lung, the diffusion coefficient and an effective airspace radius, $r_{\mathrm{AiDA}}$:

$$r_{\mathrm{AiDA}} = 2.89\sqrt{\mathrm{Dt}_{1/2}} \qquad \textbf{Equation 1.}$$

The model, which was originally derived by Goldberg in 1981 [104] is based on solving the Fokker-Plank equation (Eqn. 2), which describes how particle concentrations change over time as a function of gravitational forces and Brownian motion, for a simplified geometric approximation of lung tissue, described as infinitely long tubes or spheres. The Fokker-Planck equation can be written as:

$$\frac{\partial f}{\partial t} + V_s\,\nabla f - D\nabla^2 f = 0 \qquad \textbf{Equation 2.}$$

In this equation $\nabla$ is the volume gradient, $f = f(\mathbf{r},t)$ the concentration of particles in position $\mathbf{r}$ at time $t$, $V_s$ is the particle settling velocity and $D$ is the diffusion coefficient for the particles.

$V_s$ and $D$ are dependent on particle size, and have to be calculated for all particles included in the analysis. In the case of AiDA, monodisperse nanoparticles are used, and thus the equation can be simplified. As the aerosol is monodisperse, all particles have approximately the same properties, and $D$ is constant. Further, for nanoparticles in the size span used in AiDA (<100 nm), as seen in Figure 5, the displacement by sedimentation is negligible in

comparison to diffusion, thus the second term, describing sedimentation, can be excluded which results in a simplification:

$$\frac{\partial f}{\partial t} - D\nabla^2 f = 0$$ **Equation 3.**

In the description of the lung, its geometry is approximated as an infinitely long tube. It can be noted that, when calculating particle recovery, this mathematical description also satisfies a geometry consisting of equally sized spheres with the same radius.

The initial state at $t = 0$, $f = f(\mathbf{r},0)$ can then be described by the Fokker-Planck equation, combined with boundary conditions defined by the geometry described above (infinitely long tube with radius = $a$), a concentration condition $f = 0$ at the perfectly absorbing walls of the geometry, and an initial concentration profile. The recovery of particles after a given time $t$ can then be determined by solving and integrating the equation with polar coordinates $\boldsymbol{r} = (r, \theta)$:

$$R(t) = \frac{\int_{-\pi}^{\pi}\int_{0}^{a} f(\boldsymbol{r},\theta,t) r \, d\boldsymbol{r} \, d\theta}{\int_{-\pi}^{\pi}\int_{0}^{a} f_0(\boldsymbol{r},\theta,0) r \, d\boldsymbol{r} \, d\theta}$$ **Equation 4.**

Here $a$ is the radius of the hypothetical tubes approximating lung structure.

In the above described approximation, $R$(t) can be expressed and solved for a single dimensionless parameter $\Delta = Dt/a^2$. Particular solutions can be determined for two likely cases, an initially spatially uniform concentration profile and radially symmetric parabolic initial concentration profile as seen in Figure 8 where the solutions are plotted for a tube with ($a$ = 290 μm).

**Equation 5**: Uniform initial Concentration profile

$$R = 4\sum_{m=1}^{\infty} \frac{1}{k_{0,m}^2} e^{-k_{0,m}^2 \Delta}$$

**Equation 6**: Radially symmetric parabolic initial conc. Profile, $f_0(r)=a^2-r^2$

$$R = 32\sum_{m=1}^{\infty} \frac{1}{k_{0,m}^4} e^{-k_{0,m}^2 \Delta}$$

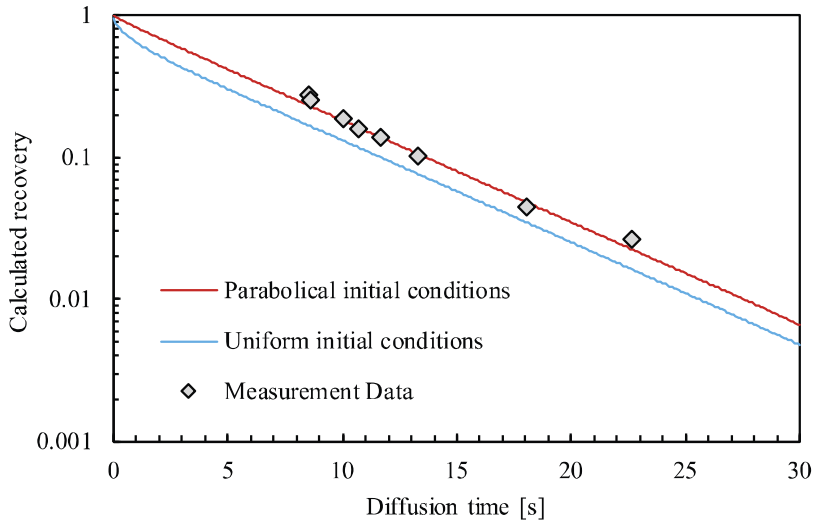


**Figure 8:**
The solution for parabolic initial concentration profile and uniform initial concentration profile for a structure with the characteristic dimension 290 µm, and 50 nm particles, compared to data from particle recovery measurements on a subject with the same airspace dimension, (normalized by dividing the measured recovery with $R_0$ to account for particle losses not caused by diffusion). The sums were approximated by the first 8 terms of the sums in Equations 5 and 6.

In these solutions, Δ is the single dimensionless parameter ($Dt/a^2$), and $k_{0,m}$ is the m:th consecutive zero for the Bessel function of the first kind $J_0(x)$. Bessel functions are solutions to the Bessel differential equation.

It is seen that the calculated deposition rate (the slope of the curve) is effectively the same after a few seconds, regardless of initial conditions. There is an initial fast, multi exponential phase followed by a slower single exponential phase that behaves like a single exponential function. After a few seconds the first term will dominate, and the relation can thus be approximated by as single exponential function. Some experimental data are displayed in the figure, normalized to $R_0$, as the expression only accounts for diffusional losses during the static phase of the experiment. It can be seen that the parabolic initial condition fit the data more closely, which is as expected to be for the distal lung.

By simplifying the solution for parabolic initial conditions by approximating it to the first exponential term of the sum (Equation 5), a relation between the particle decay rate (the particle half life time, which can be determined from recovery data) and the characteristic dimension of the geometry ($a = r_{AiDA}$) can be estimated as:

$$\frac{k_{0,1}^2 D t_{1/2}}{r_{AiDA}^2} = \ln 2 \qquad \textbf{Equation 7.}$$

This results in the relation between particle half life time in the lung and effective airspace radius as:

$$r_{\mathrm{AiDA}} = 2.89\sqrt{\mathrm{Dt}_{1/2}} \qquad \textbf{(Equation 1.)}$$

## Airspace Dimension Assessment Instrumentation

The AiDA set-up is described in detail in the included papers, particularly in Paper II. The instrument is constructed according to design criteria for lung deposition measurement systems as reviewed by Löndahl et al. [59], and has been developed in several steps to its current form. The instrument should be considered as a functional prototype with potential for improvements.

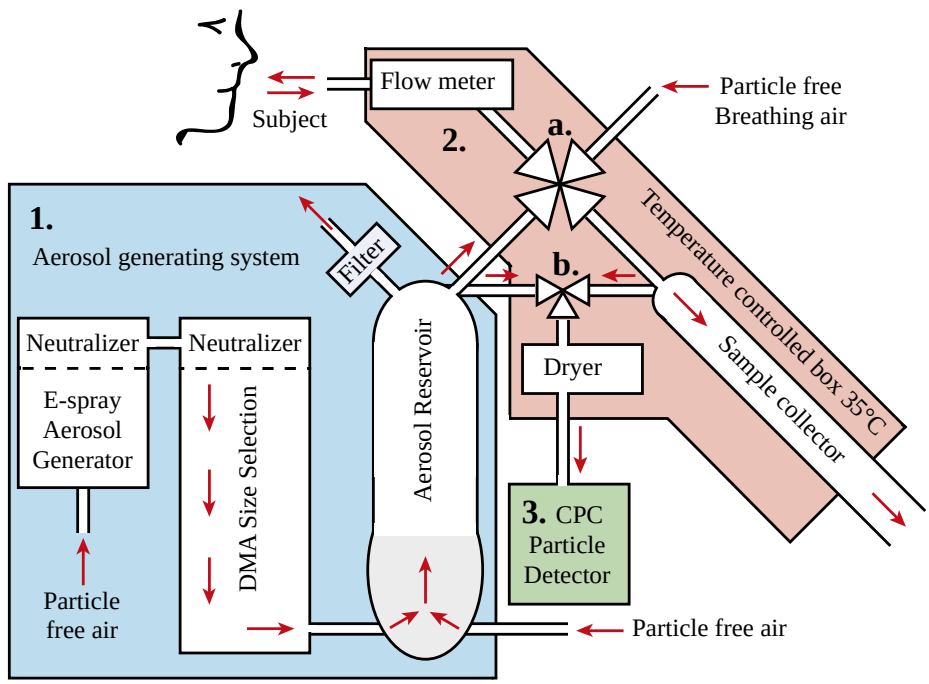


**Figure 9:**
The AiDA instrument. 1 (Blue): The aerosol generation and conditioning system consists of an electrospray aerosol generator, a differential mobility analyser (DMA) for particle size control and a supply of particle free dilution air. 2 (Red): The inhalation and sampling system consists of a flow meter and a computer controlled 4-way valve (a.) that controls the

flow of test aerosol to the subject and samples of exhaled aerosol to the sample collector. 3 (Green): A 3-way valve (b.) directs the flow to the aerosol analysis system which determines particle number concentration of the inhaled test aerosol before the inhalation and in the exhaled samples by means of a condensation particle counter (CPC).

The instrument consists of three sub systems: 1.) a system for test aerosol generation and conditioning, 2.) an inhalation system for administering the test aerosol to the subject and collection of breath samples and 3.) a system for aerosol analysis. An overview of the system is shown in Figure 9. Each of the systems have specific challenges and some of them will be briefly commented on in the following sections.

## Aerosol generation

There are not many technologies for production of monodisperse nano aerosols suitable for the AiDA technique. The requirements for the test aerosol are discussed in Paper I and II. The aerosol should be highly monodisperse, the particles should be hydrophobic to avoid hygroscopic growth in the lung, have low charge and have low toxicity. The setup with an electrospray aerosol generator (EAG) in combination with a differential mobility analyser accomplishes this. Unfortunately, electrospraying is a complicated process, and the operation of the EAG has been a limiting factor during some parts of the project. One important step in the development of the methodology of the project was a modification to the EAG by replacing the commercially available capillaries for the instrument with in-house laser cut capillaries of a larger calibre. This facilitated a more stable performance of the EAG and decreased the cost of measurements significantly.

## Inhalation system

The inhalation system controls the administration of particle free air or test aerosol to the test subject, and controls the volumetric sample depth from which the exhaled breath samples are collected. The main challenges encountered during the project regarding this sub system is the time resolution and precision needed for accurate sampling. The normal peak expiratory flow (PEF) value for a 190 cm 35-year-old male is more than 660 lpm [109]. The pneumoptachograph flow meter used in the AiDA system is set to a time resolution of 100 Hz. Thus, the highest possible volume resolution the instrument can be expected to achieve at this high flow rate is approximately 110 mL.

Another challenge related to the inhalation system is the particle losses that occur in the instrument itself. A description of how the particle losses were estimated and are accounted for can be found in Paper II.

The inhalation system is heated to approximately 36° C to avoid condensation in the system and constructed of electrically conducting materials to avoid build-up of static electricity, which could cause electrostatic particle losses in the instrument.

### Aerosol analysis

The aerosol analysis was performed with a condensation particle counter (CPC). Three different models were used during the project, and all performed equally. One subject regularly performed a standard measurement protocol with the AiDA instrument to check the consistency of the measurements (see Figure 18). Some minor variations in detection efficiency can be expected between individual CPCs, but as AiDA is performed by relative measurements and both the inhaled reference particle concentration and the exhaled samples are measured with the same instrument, individual variations of CPC models are not expected to cause uncertainties. This is given that the CPCs have a linear response, which can be assumed for the particle sizes and concentrations used during the project.

Currently the only available alternative for concentration measurements of airborne nanoparticles with sufficient time resolution and precision for AiDA measurements is the use of electrometers. Preliminary experiments with an electrometer has been performed with positive results, and indicates that an electrometer could possibly be used in future versions of the AiDA instrument. Electrometers have the advantages that they are less expensive than CPCs, does not require butanol, have high time resolution and can be placed with more flexibility in an instrument, compared with a CPC which have a butanol saturator which set restrictions on how it can be incorporated into an instrument.

## Clinical techniques

To be able to relate the AiDA measurements to actual lung properties and subject characteristics, a number of established clinical techniques were used during the studies. A brief description of the techniques is given in the following section.

### Pulmonary function tests

The most widely used assessment of lung function is spirometry. This includes the measurement of lung volumes and flows during different breathing manoeuvres performed by the subject, through a mouthpiece connected to a sensitive flow meter. The most commonly used breathing pattern and some of the measured volumes are shown in Figure 10 [110], and parameters that usually are recorded are shown in Table 3. [110]. An

evaluation of this information and normalization to subject characteristics such as height, age and gender can be used to evaluate the patient's lung function. The technique is used to detect impairment of lung function, such as airflow obstruction but does not provide specific information about the cause of the impairment.

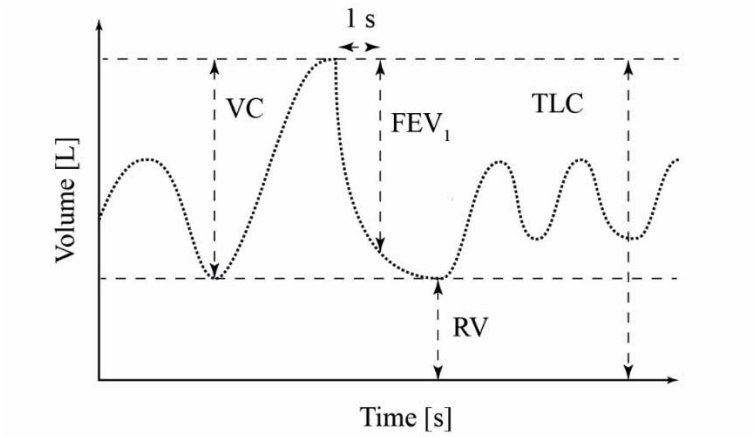


**Figure 10:**
Definition of some pulmonary function test variables commonly used for lung function assessment [108]. The definitions are given in Table 3.

**Table 3:**
Definition of some pulmonary function test variables commonly used for lung function assessment [108].

| Parameter | Definition | Symbol | Unit |
|---|---|---|---|
| Total Lung Capacity | Total lung gas volume at maximum inspiration. | TLC | L |
| Residual volume | The air volume that remains in the lung at maximum expiration. | RV | L |
| Vital capacity | Maximum volume which can be exhaled from TLC. | VC | L |
| Forced expiratory volume in 1 s | Volume which can be exhaled in a forced manoeuvre from TLC in 1 s. | $FEV_1$ | L |
| Relative expiratory capacity in 1 s | Forced expiratory volume in 1 s expressed as % of vital capacity. | $FEV_1/VC$ | % |
| Peak expiratory flow | Peak flow at maximum expiratory effort. | PEF | L/s |

## Diffusing capacity for carbon monoxide in the lung $D_{L,CO}$

The diffusing capacity for carbon monoxide in the lung, ($D_{L,CO}$), gives information about the diffusion properties of the lung. Carbon monoxide is used as its transfer from alveolar gas to blood is diffusion limited and because it has high affinity to haemoglobin. By letting a patient inhale a mixture containing a poorly soluble tracer gas (such as He) and a low (0.3%) concentration of carbon monoxide and measuring the concentration before and

after a standardized breathing manoeuvre the diffusion capacity of carbon monoxide can be determined [107]. The poorly soluble tracer gas will be diluted but not pass to the blood while the carbon monoxide concentration will decrease both by dilution and by absorption through the alveolar wall. By examining the proportions of carbon monoxide and inert tracer gas, $D_{L,CO}$ can be determined.

$D_{L,CO}$ gives indirect information about the lungs capacity to oxygenate the blood but is affected by several different parameters, such as haemoglobin values [111], carbon monoxide saturation of the blood or altered alveolar membranes [112]. A decreased $D_{L,CO}$, when occurring in combination with airflow obstruction, indicates the presence of emphysema, but is, as stated previously, also sensitive to other conditions. $D_{L,CO}$ was used in Paper III and IV, and for the subjects participating in SCAPIS.

## Computed Tomography and Magnetic Resonance Imaging

High Resolution Computed Tomography is currently considered to be one of the most powerful techniques to detect and quantify pulmonary emphysema in vivo [113-117]. The technique generates 3D images which have to be analysed by a specialist. Emphysema can be detected and quantified by subjective visual grading or by objective densitometry measurements [118] such as mean lung density (MLD), Low Attenuation Volume ($LAV_{\%}$), [119], or 15$^{th}$ percentile lung density ($PD_{15}$) [120]. The use of CT to diagnose emphysema has to be justified by benefits to the patient outweighing the risks from the exposure to radiation. In contrast to CT, AiDA has the advantage of not entailing a radiation dose. The exposure to radiation, and high costs, limits the use of CT in investigation and diagnosis of COPD and pulmonary emphysema. CT was used to assess the presence of pulmonary emphysema in Paper III, and the results of this analysis are also described in detail in a related publication not included in this thesis [14]. For the 671 subjects participating in the SCAPIS study where AiDA measurements were carried out, CT data is also available although not yet fully analysed.

Magnetic Resonance Imaging (MRI) is a powerful technique to produce 3D images with high contrast resolution but limited spatial resolution compared to CT. Unfortunately even at end expiration, lung density, and therefore the water/proton content of the lung is low and this limits the use of standard MRI for the lungs [121]. To enhance the capabilities of MRI for lung examinations hyperpolarized noble gases can be used as contrast agents [121-124]. $^{3}$He and $^{129}$Xe are the most commonly used gases [121]. It has been shown that that AiDA reflects lung density quantified by MRI (Spearman´s Rho = 0.55, $p < 0.009$) [108].

## Forced Oscillation Technique and Impulse Oscillometry

The Forced Oscillation Technique (FOT) and Impulse Oscillometry (IOS) are techniques that measure mechanical properties of the lung by means of the respiratory impedance ($Z_{rs}$) resulting from applied sound waves. FOT was developed in 1956 [125], and IOS is a later development of FOT [126], characterized by the use of an impulse-shaped, time-discrete external forcing signal, which enables efficient measurements of respiratory impedance spectra [127].

In these techniques the subjects breathe normally through a mouthpiece connected to a loudspeaker that generates soundwaves. The soundwaves travel through the respiratory system, and the respiratory impedance is determined from the ratio of resulting pressure and flow, which is recorded and separated from the tidal breathing by signal processing. The respiratory impedance is usually measured at multiple frequencies between 5 – 35 Hz. Low frequency oscillations travel deep into the lung and are believed to give information from the whole respiratory system while higher frequencies don't travel as far and thus give information mainly from the larger, more central airways. The respiratory impedance is a complex signal and can be interpreted as the sum of all forces (resistance and reactance) opposing the applied pressure impulses, and can be used to derive information about the mechanical properties of the lung. The in-phase (real) component of the complex impedance signal is interpreted as respiratory resistance, ($R_{rs}$), and gives information about the forward pressure of the conducting airways.

The out of phase (imaginary) component is termed respiratory reactance, ($X_{rs}$), and can be interpreted as the sum of inertive forces, resulting in movements of the air column in the respiratory system, and capacitive forces, which reflect the elasticity of the airways. At high frequencies capacitive pressure losses are high and the inertive forces dominate, while the opposite is true for low frequencies. At some point, the inertive and capacitive forces are equal, and thus $X_{rs}$ is zero. The frequency where this occurs is called the resonant frequency, ($F_{res}$) and is a convenient variable to distinguish between low frequency and high frequency $X_{rs}$. Below $F_{res}$, the elastic component dominates, while above this frequency, inertance dominates. The integral of $X_{rs}$ below $F_{res}$ is called the reactance area (Ax) or the Goldman triangle after its inventor, and is a common parameter used to interpret IOS and FOT. Ax has been shown to provide information about peripheral airway obstruction [127].

FOT and IOS are attractive techniques as they are easy to perform and provide clinical relevant information about the lung, but there are still some aspects of the techniques that are not yet fully understood, and there is no consensus as to how they should be interpreted in clinical practice [128].

IOS was used to measure respiratory resistance ($R_5$ and $R_{20}$), respiratory reactance ($X_5$), $F_{res}$ and Ax for a large group (n = 736) research subjects in SCAPIS, and some preliminary results are presented in this thesis.

# Results and Discussion

The main results of this thesis are presented in detail in the included papers. Here follows a brief summary of the most important findings, a preliminary overview of the data collected within SCAPIS for future publications. To clarify why different approaches to the AiDA technique have been used in the different studies, a short description of how the method has evolved is included. The results are discussed and compared to general criteria for diagnostic techniques. Finally practical recommendations based on experience are given for working with the AiDA technique.

## Summary of main results in the included papers

### Paper I

A simple model for assessment of effective airspace radii, $r_{\mathrm{AiDA}}$, by measurements of inhaled nanoparticles was derived (see also "Methods section"):

$$r_{\mathrm{AiDA}} = 2.89\sqrt{D t_{½}} \qquad \textbf{(Equation 1.)}$$

Here $D$ is the diffusion coefficient and $t_{½}$ the particle half-life time in the respiratory tract. It was estimated that the particle range 40-100 nm is optimal for AiDA. Based on review of previous publications and theoretical considerations, it was concluded that assessment of distal airspace dimensions with nanoparticles was likely to offer several advantages compared to previous aerosol based methods. Among the suggested advantages are: (A) The ability to use normal breathing flowrates and (B) the ability to apply the technique at full TLC instead of at a controlled % of TLC. (C) An increased sensitivity to enlarged distal airspaces. (D) A simpler interpretation of the derived effective airspace radii as particle deposition was expected to be exclusively from diffusion. (E) Less deposited mass in the lungs compared to previous techniques. And finally (F) Better penetration of inhaled nanoparticles into diseased and poorly ventilated regions of the lung compared to larger particles.

## Paper II

An instrument for measurements of lung deposition of nanoparticles was described and characterized on a group of 7 healthy subjects. Measurements with the instrument showed that the measurement precision of the instrument was 26-50 times higher than individual variations, even for a small, homogeneous group of healthy subjects. This means that the instrument is capable of measuring subject specific properties. It was also shown that the measured particle recovery varied with residence time in the lung and particle size as expected from the theory of diffusion, and that the measurement was in reasonable agreement with the results from calculations with the MPPD model. It was shown that the particle recovery is mostly determined by the residence time in the lung and was less affected by respiratory flow rates in the range 1-10 L/s, as predicted by Paper I. A model was also given for adjusting measurements for particle losses in the instrument. Repeated measurements on one subject at 15 different occasions over a one-year time period showed that the measurements were reproducible (coefficient of variation = 0.056). The instrument fulfils the requirements for measurements suitable for the AiDA method and is, to our knowledge, the only instrument capable of measuring lung deposition efficiency of nanoparticles on human subjects in a single breath.

## Paper III

It was shown that *DF* measured with the AiDA instrument was significantly lower for subjects with diagnosed COPD compared to healthy subjects ($p < 0.01$-$0$-$001$), but no significant difference was found between healthy never-smokers and asymptomatic smokers. CT was used to confirm if the COPD group had emphysema, and the CT results are described in more detail for a subset of the subjects in a related publication [14]. Correlations were found between *DF* and pulmonary function tests when all subjects were included (Table 4). It was shown that *DF* measured with the instrument correlated with the severity of emphysema for COPD subjects, assessed with $D_{L,CO}$ (Pearson's $r = 0.80$-$0.85$, $p < 0.002$), but no correlation was found for the healthy subjects. A negative correlation was found between $FEV_1$ and *DF* for the healthy group which was strongest for 100 nm particles (Pearson's $r = -0.73$, $p = 0.001$).

The results imply that AiDA has sensitivity for lung morphology. No correlations were found between *DF* and sex, age, weight, height or VC included subjects in this study. An important observation in this study was also that AiDA measurements could be performed on subjects with severe respiratory disease, but that special care had to be taken to instruct and encourage them.

**Table 4:**
Correlations found between lung deposition fraction and pulmonary function tests for the 48 subjects in Paper III.

| | | ***DF*** **50 nm 1300 mL** | ***DF*** **50 nm 1800 mL** | ***DF*** **100 nm 1800 mL** |
|---|---|---|---|---|
| **$FEV_{1\%pred}$** | Pearson's r | 0.32* | 0.39** | 0.36** |
| | p | 0.03 | 0.006 | 0.013 |
| **$FEV_1/VC_{\%pred}$** | Pearson's r | 0.45** | 0.53*** | 0.57*** |
| | p | 0.001 | 0.0001 | 0.00002 |
| ***$DL_{,CO\ \%pred}$*** † | Pearson's r | 0.49*** | 0.57*** | 0.52*** |
| | p | 0.0005 | 0.00003 | 0.0002 |

† $n$ = 46, $D_{L,CO}$ data for two subjects is missing.
***** Significance < 0.05 level.
****** Significance < 0.01 level.
******* Significance < 0.001 level.

## Paper IV

$R_0$ and $r_{AiDA}$ was measured for volumetric sample depths ranging from close to anatomical dead space to the distal lung on 19 healthy subjects (aged 17-67 yrs.) using the model derived in Paper I. The study contains the most detailed measurements performed with the AiDA method and can be used to design future AiDA measurement protocols. It was found that $r_{AiDA}$ varied with volumetric sample depth in a way that was consistent with established lung models for the same volumetric sample depths, with larger values for sample depths < 1000 mL and then smaller and more uniform values for sample depths > 1000 mL, as seen in Figure 11, where average values are compared with a lung model [129]. As expected from the theory presented in Paper I, the $r_{AiDA}$ values were larger than the normal arithmetic means given by lung models.

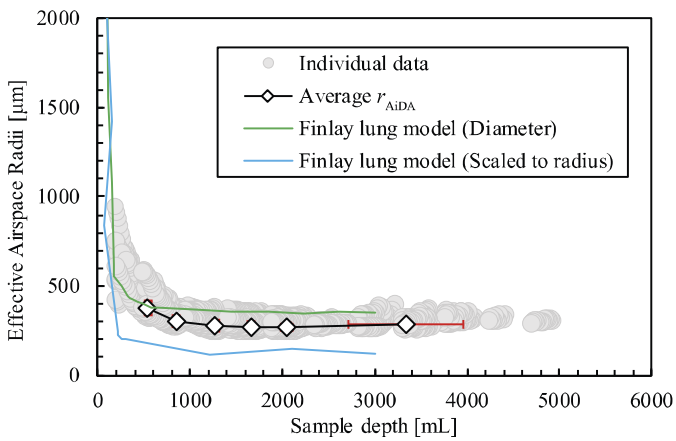


**Figure 11:**
Effective airspace radii for 19 subjects in Paper IV compared to lung model [129]. The lung model values are given as diameter (green), and also scaled to radii (blue) in this Figure. The Figure shows that the $r_{AiDA}$ values follow the general pattern expected from lung models, but are approximately a factor 2x larger than arithmetic means.

It was found that $R_0$ was highly dependent on the volumetric sample depth from which it was measured, with decreasing $R_0$ for deeper volumetric sample depths.

The derived $r_{AIDA}$ values were found to correlate both to subject characteristics such as anthropometric measurements (height $p < 0.014$ and weight $p < 0.04$) and to subject age ($p < 0.007$), which is interesting as dimensions in the distal lung have been shown to change with age [130-132]. The $r_{AIDA}$ values also correlated with lung function tests (TLC, RV, FRC, $FEV_1$/VC), but not at all volumetric sample depths. Significant correlations were only found between $R_0$ and age ($p < 0.037$), and to the transfer coefficient of carbon monoxide, ($K_{CO}$), but there were also several indications ($0.05 < p < 0.1$) for correlations with other subject characteristics and lung function tests. It can be interpreted as that the relations between $R_0$ and the other variables was too weak to be detected in a small group of homogeneous, healthy subjects. Importantly, no strong correlations were found between $r_{AIDA}$, and $R_0$ which indicate that they reflect different lung properties. Repeated measurements were performed on three subjects with up to 18 months between the measurements, and it was shown that the AiDA technique had high reproducibility. The $r_{AiDA}$ values for volumetric sample depths between 1000-2500 mL were on average within 7 µm (or 2.4 % of the average $r_{AiDA}$ value) for these three subjects.

# The evolution of AiDA methodology

The development of the AiDA technique has evolved stepwise, from construction of an instrument capable of measuring the deposition efficiency of nanoparticles in a single breath, to a method of charting the human respiratory tract with nanoparticles. Various approaches have been used in the different studies, and this section offers a short description of the evolution of the AiDA technique, and the different ways of reporting AiDA data in the included papers.

The first measurements that were performed with the AiDA instrument were simply reported as the lung deposition fraction (*DF*) or particle recovery (*R*). *DF* and *R* are calculated from a reference concentration of the inhaled aerosol, determined as the average of the particle number concentration of the test aerosol reservoir directly before the inhalation and from the average particle concentration in a relatively large exhaled sample representing air from the distal lung.

The measurements were adjusted for particle losses in the instrument, as described in Paper II, and also time normalized, as described in Paper III and a related publication [14]. At least three measurements were performed and the result was given as the average and standard deviation. AiDA measurements reported as *DF* are given in Paper III. The measurement represents an average value for a relatively large sample volume (≈500 mL),

chosen to be representable for air from the respiratory zone of the lung. The measurement is illustrated by CPC data (normalized concentration = recovery) from a typical measurement in, Figure 12:

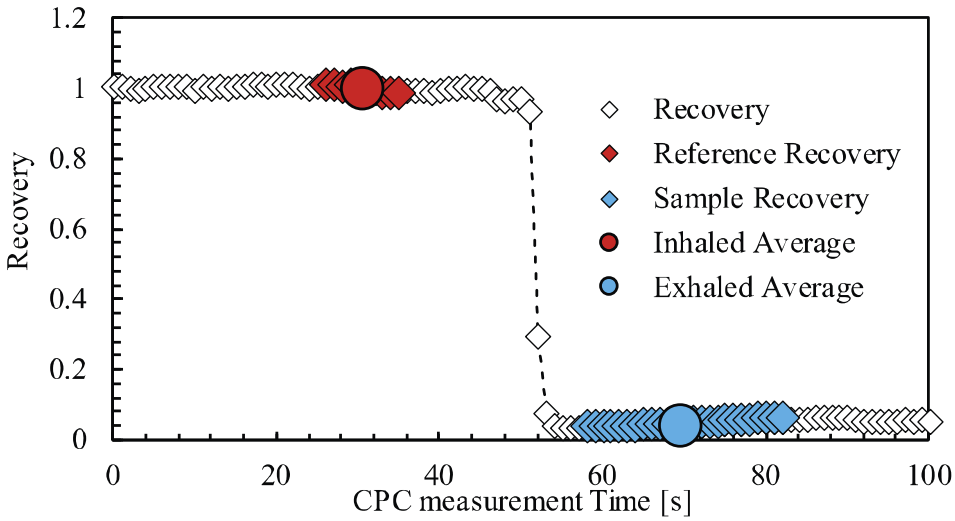


**Figure 12:**
Simple measurement of particle recovery or lung deposition fraction. Particle recovery is calculated as the fraction of the average particle concentration in the exhaled sample relative the inhaled reference concentration. The lung deposition fraction is the complement to particle recovery *DF* = 1- *R*.

The next step in the evolution of AiDA methodology was the implementation of the theoretical model for interpreting AiDA measurements, presented in Paper I, as the derived variables $R_0$ and $r_{AiDA}$, which gives more specific information. For this *R(t)* must be measured for varied breath holding times as shown in Figure 7. Before this model could be applied it was necessary to confirm that the instrumental setup was capable of producing data of sufficient quality, and to evaluate suitable test parameters. This was done by the characterization of the AiDA instrument in Paper II.

During the analysis of recovery data, it was observed that there seemed to be a concentration gradient in the sample collector, as seen in Figure 13 which shows the particle recovery signal for measurements on exhaled samples after different breath holding times but from the same sample volume.

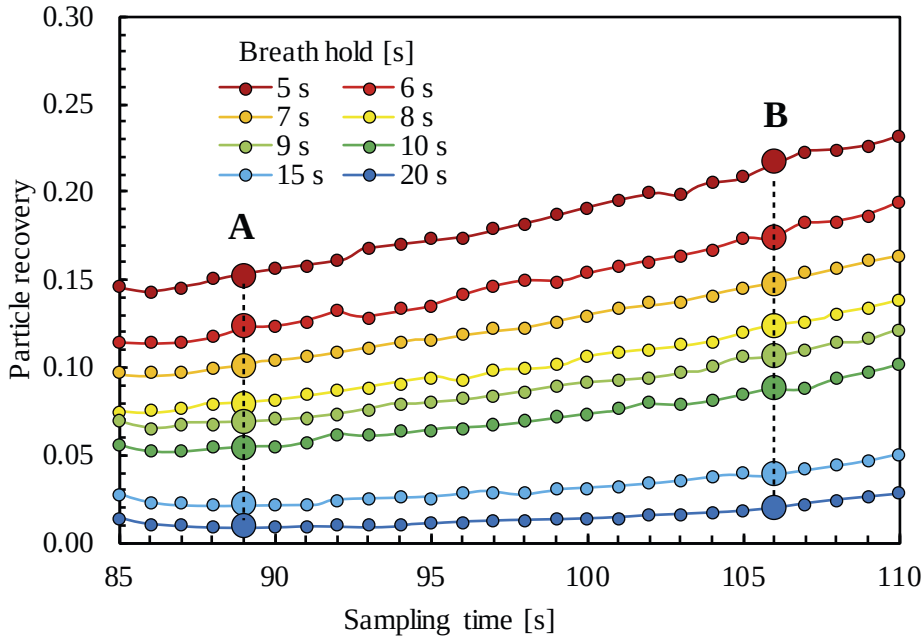


**Figure 13:**
Relative concentration (recovery) signal from the CPC after different breath holding times, 1500 mL sample depth. It seems likely that analysis of the time series constructed from the data marked at A and B could provide more detail than an average for the full samples.

This suggested that the AiDA technique may have some volumetric resolution. This was further investigated in Paper IV, where AiDA was used to measure $R_0$ and $r_{AiDA}$ for varied volumetric sample depths on a group 19 healthy subjects.

The methodology in Paper IV includes the most elaborate measurement protocol performed so far with the AiDA instrument. The measurement protocol in this study includes a total of 48 measurements of recovery with upper exhaled sample fronts from 700 – 2500 mL and a measurement at a sample depth close to VC, with breath holding times from 5 – 20 s, which enables measurement of $r_{AiDA}$ and $R_0$ from approximately 200 mL to the distal lung as shown in Figure 14:

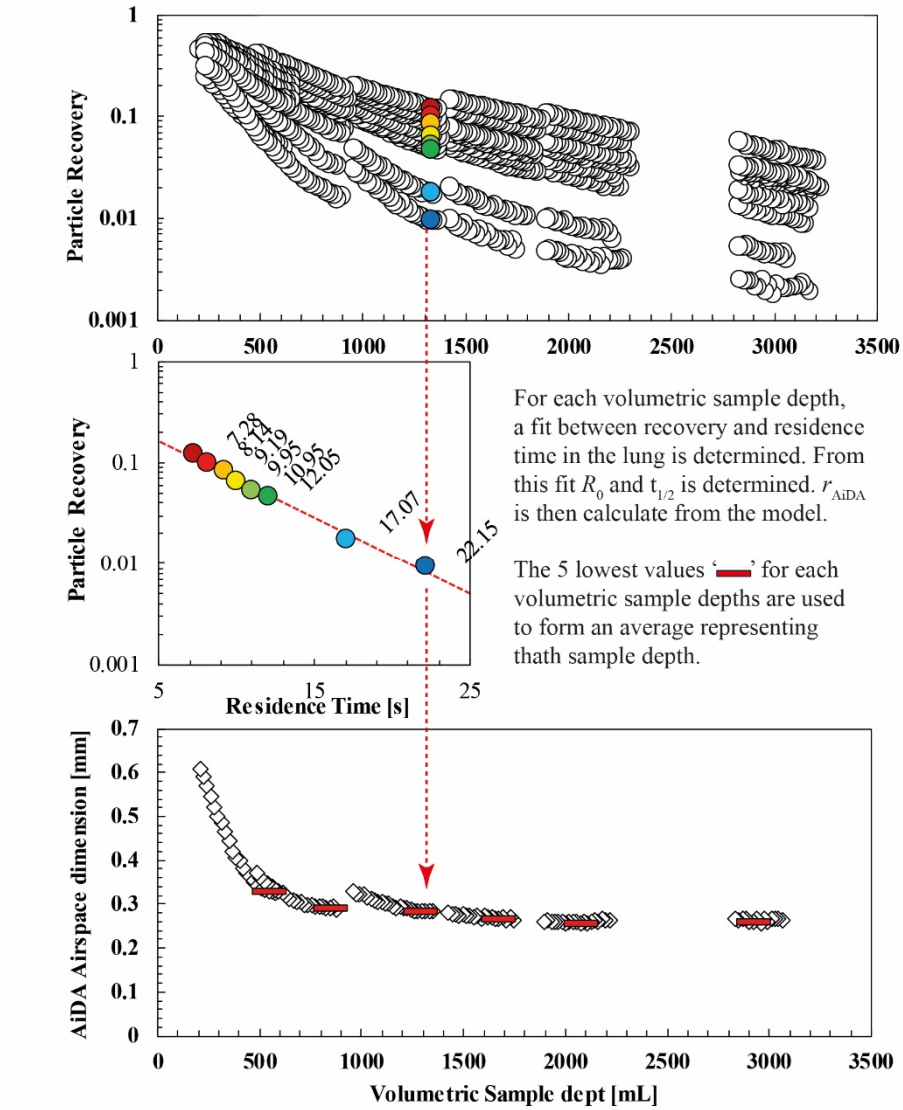


**Figure 14:**
The measurement protocol used in Paper IV. Particle recovery is measured as a function of volumetric sample depth in the lung and residence time in the lung (middle panel). For each volumetric sample depth, a curve fit can be made from which $R_0$ and particle half-life time in the lung can be determined (upper panel). From the particle half-life time in the lung, $r_{AiDA}$ can be determined for that volumetric sample depth (lower panel).

By matching the volume of samples with different residence times in the lung by adjusting for the dead space in the instrument, the calibration of the pneumoptachograph and the flow rate of the particle detector, a map of particle recovery as function of residence time in the lung and volumetric sample depth can be produced as shown in the upper panel of Figure 14. The results of Paper IV show that $r_{AiDA}$ measured at shallow sample depths (< 1000 mL) decrease rapidly until they reach a more constant value. This value is

interpreted as reflecting the more homogeneous acinar lung tissue, which makes up most of the lung volume. This is important as it indicates that measurements > 1000 mL volumetric sample depth can be assumed to reflect typical acinar airspace radii for individual subjects. Thus it is possible to obtain a measurement of typical airspace radii for a subject in a limited number of measurements at a volumetric sample depth > 1000 mL. It was also found that an average of the five lowest observed $r_{AiDA}$ values (red bars in the lower panel of Figure 14) from each nominal volumetric sample gave the best approximation of the full data set, in the lowest number of variables. Other approximations, such as the average of all data points in the sample or the average of the mid-range data of the sample resulted in measurements that over-estimated $r_{AiDA}$ compared to the full data set. The reason for this is likely to be that most measurement artefacts, e. g. low particle concentrations resulting in poor counting statistics, increase the value of $r_{AiDA}$. Thus the lowest observed values appear to be the data least affected by measurement artefacts. This observation may provide an enhancement of future analysis of AiDA measurements.

# Data collected for future publications: SCAPIS

Effective airspace radii, $r_{AiDA}$, and zero second recovery, $R_0$, was measured as described in the Methods section on 736 subjects. The data analysis is not finalized, and several publications are planned. Some preliminary data and results are presented here. Data from measurements performed within the scope of the SCAPIS study [105] are presented in Table 5-9 for 671 of the measured subjects for which all quality criteria for the AiDA measurements were fulfilled. As the analysis is not finalized, and future publications are planned, the data in Table 8 - 9 are only given qualitatively.

**Table 5:**
Subject characterization, available data for 671 mostly healthy subjects from a population based study. The spirometry and $D_{L,CO}$ values are expressed as % of reference values according to current recommendations [107, 109].

| | Unit | N | Min | Max | Mean | Std. |
|---|---|---|---|---|---|---|
| **Gender** | [M/F ] | 318/353 | | | | |
| **Age** | [yr.] | 671 | 50.1 | 65.4 | 57.4 | 4.5 |
| **Height** | [cm] | 671 | 146.0 | 199.0 | 171.6 | 9.6 |
| **Weight** | [kg] | 671 | 43.0 | 147.0 | 81.2 | 16.6 |
| **FVC (%pred.)** | [%] | 669 | 60.1 | 154.0 | 110.2 | 14.5 |
| **$FEV_1$(%pred)** | [%] | 669 | 30.2 | 152.3 | 105.8 | 15.2 |
| **$FEV_1$/VC(%pred)** | [%] | 668 | 37.5 | 135.2 | 100.7 | 10.3 |
| **$D_{L,CO}$(%pred)** | [%] | 633 | 27.5 | 155.6 | 90.8 | 13.1 |

**Table 6:**
Median, average and standard deviation for AiDA values measured with 50 nm particles from a volumetric sample depth of 1300 mL and with duplicate measurements of recovery after 5, 7 and 10 s breath holds. The values given are: $R_0$, the exponent of the exponential fits of recovery data to aerosol residence time in the lung, the coefficient of determination, r, for the fits, the particle half-life time in the lung ($t_{1/2}$) and $r_{AiDA}$.

| N = 671 | $R_0$ | Exponent | r | $t_{1/2}$ [s] | $r_{AiDA}$ [µm] |
|---|---|---|---|---|---|
| **Median** | 0.489 | 0.169 | 0.991 | 4.1 | 287 |
| **Average** | 0.492 | 0.170 | 0.988 | 4.3 | 293 |
| **Standard deviation** | 0.196 | 0.039 | 0.012 | 1.2 | 38.5 |

**Table 7:**
Impulse Oscillometry for the group, also expressed as %pred = percentage of reference value adjusted for height, age and gender [133].

| | Unit | N | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|---|---|
| **$R_5$** | [kPa/L/s] | 668 | 0.15 | 0.73 | 0.34 | 0.09 |
| **$R_{20}$** | [kPa/L/s] | 668 | 0.06 | 0.75 | 0.30 | 0.08 |
| **$X_5$** | [kPa/L/s ] | 668 | -0.42 | 0.06 | -0.09 | 0.04 |
| **Ax** | [kPa/L] | 668 | -0.02 | 3.42 | 0.28 | 0.31 |
| **$F_{res}$** | [Hz] | 668 | 5.94 | 24.84 | 10.84 | 3.23 |
| **$R_5$ (%pred)** | [%] | 668 | 62 | 282 | 116 | 28 |
| **$R_{20}$ (%pred)** | [%] | 668 | 23 | 358 | 133 | 33 |
| **$X_5$ (%pred)** | [%] | 668 | -85 | 337 | 95 | 40 |
| **Ax (%pred)** | [%] | 668 | -1344 | 4045 | 97 | 211 |
| **$F_{res}$ (%pred)** | [%] | 668 | 41 | 266 | 86 | 26 |

**Table 8:**
Non-parametric correlations (Spearman's Rho) between AiDA, subject characteristics and pulmonary function tests. Because the analysis is preliminary, and future publications are planned, the results are only given qualitatively. Significant correlations on different levels are marked by * (0.05 < p < 0.01), ** (0.01 < p < 0.001), *** (0.001 < p < 0.0001), **** (0.0001 < p < 0.00001) and ***** (p < 0.000001).

| | $R_0$ | $r_{AiDA}$ |
|---|---|---|
| **Age** | - | *** |
| **Weight** | ** | - |
| **Height** | ***** | *** |
| **Waist** | - | - |
| **FVC (%pred)** | ***** | * |
| **$FEV_1$ (%pred)** | ***** | - |
| **$FEV_1$/VC (%pred)** | - | ** |
| **$D_{L,CO}$ (%pred)** | - | *** |

**Table 9:**
Correlation between Impulse Oscillometry and AiDA. (Spearman's Rho). Because the analysis is preliminary, and future publications are planned, the results are only given qualitatively. Significant correlations on different levels are marked by * (0.05 < p < 0.01), ** (0.01 < p < 0.001), *** (0.001 < p < 0.0001), **** (0.0001 < p < 0.00001), and ***** (p < 0.000001). A (left): Measured values, B (right): % of predicted reference, adjusted for height, weight, age and gender [133], Spearman's Rho, and p values for two sided significance.

| | A: IOS | | B: IOS (%pred) | | |
|---|---|---|---|---|---|
| | $R_0$ | $r_{AIDA}$ | $R_0$ | $r_{AIDA}$ | |
| $R_5$ | ***** | * | * | - | $R_5$ (%pred) |
| $R_{20}$ | *** | - | - | - | $R_{20}$ (%pred) |
| $X_5$ | ***** | *** | ***** | - | $X_5$ (%pred) |
| Ax | ***** | ** | ***** | - | Ax (%pred) |
| $F_{res}$ | ***** | * | **** | - | $F_{res}$ (%pred) |

The $R_0$ values for the group is normal distributed, with an average value close to 0.49 and a standard deviation of 0.2. The exponent of the curve fits, which is used to determine particle half-life time in the lung and therefore $r_{AiDA}$ also appears to be normal distributed, while $r_{AiDA}$ is somewhat skewed, resembling a log-normal distribution. It appears that the $r_{AiDA}$ values are more centred around a rather narrow interval, with few subjects having $r_{AiDA}$ values smaller than average, while more subjects, including some outliers, appear to have increased values. This could be interpreted as most subjects having rather similar distal airspaces, and some subjects have enlarged airspaces, while the individual variation of $R_0$ is larger. Importantly, no significant correlation was found between $R_0$ and $r_{AiDA}$.

Comparing the $R_0$ and $r_{AiDA}$ values to subject characteristics and the result of pulmonary function tests also give interesting information (Table 8). $R_0$ was found to correlate with height and weight, while $r_{AiDA}$ was found correlate with height and age. Height is known to correlate with lung size [109]. The correlation between $R_0$ and weight and $r_{AiDA}$ and age suggests that $R_0$ and $r_{AiDA}$ give somewhat different information. Airspace dimensions are believed to change with age [130-132]. The correlations between the AiDA variables and subject characteristics (height, weight and age) are not strong (Spearman's Rho 0.11 – 0.23), but the p-values indicate that the correlations are probable ($p = 2.6 \times 10^{-09}$ - 0.004). This can be expected for a group of mostly healthy subjects in narrow age span.

Correlations between pulmonary function tests and AiDA (Table 8) also indicate similar trends. $R_0$ correlates with FVC(%pred) and $FEV_1$(%pred), while $r_{AiDA}$ correlates with VC(%pred) and $FEV_1$/VC(%pred) and $D_{L,CO}$(%pred). This suggest that both variables are affected by lung size, but that $R_0$ gives information about properties more related to body size and respiratory flows, while $r_{AiDA}$ gives information about properties related to the more distal lung, and also obstruction, as suggested by the correlation with $FEV_1$/VC(%pred) and

$D_{L,CO}$(%pred). Analysis of the CT data is not yet complete, but currently available data indicate that both $R_0$ and $r_{AiDA}$ also have association to CT densitometry.

$R_0$ correlated to all IOS variables with high significance (Table 9). When the IOS variables were expressed as % of reference values [133] the significance for correlations between $R_0$ and respiratory resistance ($R_5$, $R_{20}$) were much lower, while correlations to the other variables were still highly significant. The effective airspace radii, $r_{AiDA}$, correlated with lower significance to some of the IOS variables, especially $X_5$, but when the IOS measurements were expressed as % of reference values, there was no significant correlation between the variables. The stronger correlation for $R_0$ than $r_{AiDA}$ indicate that IOS gives information mainly about the small conducting airways, since $r_{AiDA}$ most likely is determined by properties of the more distal lung.

# Evaluation of AiDA as a future diagnostic technique

The appropriate criteria for the evaluation of a diagnostic test depends on the purpose of the test, and how it is intended to be applied. The ASSURED criteria is recommended by WHO for resource-constrained settings. ASSURED stands for Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users [134]. Another set of seven criteria, which is often used to assess the value of diagnostic screening tests, is given by Cochrane et al. [11]: Simplicity, Acceptability, Accuracy, Cost, Precision (repeatability), Sensitivity and Specificity. Here follows an evaluation of AiDA in its current stage as a potential diagnostic technique in relation to the Cochrane criteria.

# Simplicity and Acceptability

Simplicity here refers to how easy a test is to administer for the personnel performing the test. This includes factors such as the required training and skill for the operator, preparations that has to be made before the test and time it takes to instruct and conduct the test on a subject. Acceptability refers to the experience of the subject on which the test is performed on, and how much effort it takes to participate in the test.

### Practical application of the AiDA technique

The AiDA technique is performed very similar to the measurement of the diffusing capacity of the lung for carbon monoxide. As such it can be regarded as fairly simple to administer. However, the current instrumentation is somewhat more complicated to operate than most

commercial lung testing equipment. Several individuals have been involved in the measurements, and most operators have been able to perform adequate measurements after just a few hours training followed by some time of supervised work with the instrument. The most challenging task involved in the measurement procedure has been operation of the electrospray aerosol generator.

Most of the more than 1000 studied subjects have been able to perform standard AiDA measurement protocols of 6 – 9 breathing manoeuvres without any noticeable discomfort. This shows that the AiDA technique can be performed for a wide range of subjects including patients with severe respiratory disease. The main practical limitation experienced when performing measurements on untrained subjects was that some of the subjects with more severe respiratory disease had problems to perform the breathing pattern in sufficiently short time. In particular, to exhale a sufficient sample volume in short time compared to the breath-hold was challenging for some subjects.

## Data quality control for AiDA

Final quality criteria for AiDA measurements are not yet established. For the measurements in Paper III, two main criteria for acceptable measurements were: 1) that the subjects inhaled > 70% VC of the test aerosol and 2) that they performed the full breathing manoeuvre in less than 17 seconds (including the 10 s breath-hold). Prolonged breathing manoeuvres can to some extent be accounted for in the data analysis, but uncertainty in the measurements increase considerably when the aerosol residence time in the lung during the breath hold is short in comparison to the inhalation and exhalation time, or if the breathing manoeuvres are uneven.

When measurements were performed at several breath-holding times, as in Paper IV and SCAPIS, the main criteria for a successful measurement was the degree of correlation between the observed recovery data and the corresponding residence time of the aerosol in the lung. Data were considered acceptable when coefficient of determination, r, was larger than 0.95. It was also required that the subject fulfilled the breathing manoeuvre according to the operator's instructions.

For the SCAPIS study (n = 736), the success rate was approximately 91.2% (Figure 15). Of the measurements that did not fulfil the criteria for a successful measurement 4.5% were complete, but with $r < 0.95$ and 4.3 % of the measurements generated insufficient data for analysis because of other problems such as instrumental problems or data corruption errors. For successful measurements r was on average >0.99 (Table 6).

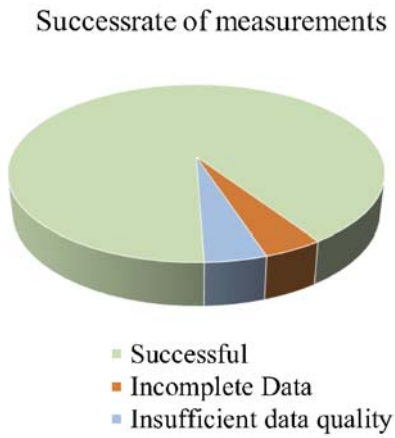


**Figure 15:**
The approximated success rate of AiDA measurements based on 736 subjects performing AiDA measurements under the scope of SCAPIS at 1300 mL sample depth and duplicate measurements at 5, 7, and 10 s breath holds.

# Accuracy

Accuracy refers to the performance of the test itself; that the test gives accurate information about the attribute the test is supposed to measure. To avoid conceptual confusion, it should be clarified that the discussion will refer to both accuracy and the related term validity. While accuracy describes how close the measurements are to true values, validity refers to if the applied method is appropriate in addressing the aim of the measurement, or more simply put: if the performed measurement give information about what was intended to be measured. Different AiDA measurements have been performed and presented in the papers, and the reported AiDA variables, particle deposition assessed as particle recovery or lung deposition fraction, zero second recovery and effective airspace radii will be discussed separately.

## Accuracy for measurements of particle recovery and *DF*

The measured particle recovery, *R*, or deposition fraction, *DF* (=1-*R*), with AiDA are in agreement with most previous experimental findings and lung deposition models [40, 41, 62, 67]. It was shown that smaller particles deposited with much higher efficiency than larger particles, as expected from both theory and previous deposition studies [67]. It was shown in Paper II that for measurements performed at different flow rates, it was still the residence time in the lung that was dominant in determining particle recovery, and not flow rate. This is in agreement with theory presented in Paper I.

In Paper II, time normalized particle recovery after 10 s residence time in the lung was compared to predicted lung deposition for different particle sizes by the Multiple-path particle dosimetry model (MPPD) [135]. The average measured particle recovery were in reasonable agreement with the modelling. For 50 nm particles, the average particle recovery was lower (44 % of the MPPD result) than the modelling results, for 75 nm particles, the average was more in line with the modelling result (81 %), and for 100 nm particles, the results agreed well (104% of the MPPD result). This may be explained by the fact that the MPPD model was developed for tidal breathing, and thus the AIDA breathing manoeuvre could not be reproduced exactly by the model. Further, the MPPD model is based on an average lung, and does not account for individual variabilities apart from lung volume.

In Paper III it was found that *DF* was decreased for subjects with diagnosed COPD, which is in agreement with the expectations from AIDA theory, that particle deposition is decreased for enlarged airspaces. It is also in agreement with previous experimental studies, although these include few subjects [43, 44, 46]. The strong correlation found in Paper III between *DF* and $D_{L,CO}$ for the diseased group supports the interpretation that particle deposition as measured with the AiDA instrument reflects lung morphology is accurate.

In summary, measurements of particle recovery and deposition fraction with the AiDA instrument are in agreement with the expectations from previous experiments and models and correlates to lung function tests as could be expected from theory. This supports reasonable accuracy for measurements of recovery and deposition fraction with the AiDA instrument.

### Accuracy for the zero second recovery, $R_0$

The zero second recovery, $R_0$, is assumed to reflect particle deposition in mainly the small conducting airways during the dynamic phase of the measurement. Accuracy of $R_0$ is difficult to evaluate, as there are no reference data and since the physical interpretation of the variable remains partly unclear (although we have good reason to believe it is related to deposition in small airways). Especially for subjects with altered lung structure, values for $R_0$ are unavailable other than those measured by AiDA. $R_0$ values should in theory be possible between 0 – 1, where 0 means that all particles deposit during the dynamic phase of the measurement and 1 that no particles deposit, but for some rare cases $R_0$ values higher than 1 has been observed, which is a result of the curve fit although obviously is inaccurate.

$R_0$ values for 19 subjects at varied volumetric sample depths are shown in Figure 16, and reported in Paper IV and in the preliminary data (Table 5, 7-8), some data is also reported in a related publications [108]. A decreased $R_0$ for deep inhalations is in agreement with the expectation, as a larger fraction of the aerosol flow occur in the distal airspaces. $R_0$ values determined for sample depths ≥ 1000 mL were most consistent. It was observed that the variability between subjects was relatively large compared for effective airspace radii, and

that the inter subject variability was much larger than the intra subject variability (Figure 16), which suggest that measurement of $R_0$ accurately reflects subject individual characteristics, or at least that the methodology is valid. As previously described, $R_0$ correlated with fundamental subject characteristics, lung function tests and IOS in a way consistent with our theoretical interpretation.

In summary, it seems likely that the applied method to measure $R_0$ is valid, and that $R_0$ can be measured fairly accurately. It also seems likely that $R_0$ reflect lung properties that may be clinically relevant. It should be noted that $R_0$ varies significantly with volumetric sample depth, and probably should be analysed normalized to % of VC or other subject characteristics. $R_0$ has only been reported for AiDA measurements with 50 nm particles, and future studies with varied particle sizes and more controlled breathing patterns with controlled respiratory flow rates would be valuable to better understand more about this variable. Development of AiDA "bolus" methods may also be of interest for the understanding of $R_0$.

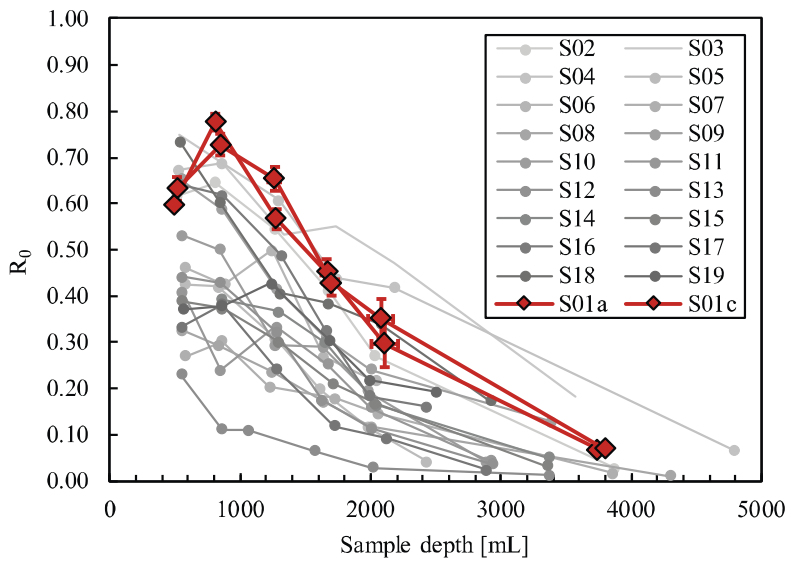


**Figure 16:**
$R_0$ values for 19 individuals in Paper IV at varied volumetric sample depths. Duplicate measurements for one subject (red) performed with 10 months between the measurements show that the reproducibility of $R_0$ is high, it also illustrates that the measurement precision is higher than the inter subject variations, even for a healthy group.

## Accuracy for effective airspace radii, $r_{AiDA}$

The effective airspace radius, $r_{AIDA}$, is the root mean square distance to a surface in the lung where the test aerosol has resided during the breath hold. As it is a root mean square value it is expected to be larger than the arithmetic means given by lung models [129, 136-138],

and also weighted toward larger airspaces, as discussed in Paper I. The accuracy of $r_{AIDA}$ is depending on the determination of the particle half-life time, as discussed in previous sections.

The $r_{AIDA}$ values were roughly a factor 2x larger than the arithmetic means given by comparable lung models (Figure 11). This is as expected, as lung models based on histological measurements give average values for each generation of the tracheobronchial tree [139], while $r_{AIDA}$ is the root mean square of all distances to surfaces, including several generation of respiratory bronchioles, alveolar ducts and alveoli in the distal lung. As such it seems to provide accurate information about the lung, especially when measured at volumetric sample depths > 1000 mL.

In summary, $r_{AIDA}$ measured on volumetric sample depths > 1000 mL is likely a valid measurement of the dimensions of the distal airspaces, expressed as a root mean square radius, and as such it can be considered accurate.

## Cost efficiency of AiDA

Before the AiDA technique is fully developed and adapted for clinical use it is not possible to assess the true cost of performing AiDA measurements or the value of the information that can be derived from the technique. It can, however, be estimated that an instrument can be produced at similar price as equipment for comparable techniques, such as $D_{L,CO}$. The construction of an instrument for AIDA measurements is much less expensive than production of a system for CT or MRI. At the current stage, the prototype of the instrument used in the presented studies include some expensive components, especially the particle generator and the particle detector, but there are more cost efficient alternatives that may be used. Cost efficiency finally has to be related to the value of the information that can be derived: if the technique is able to give information not easily obtained by other means, such as information similar to that of the apparent diffusion coefficients measured with MRI [140, 141], it is extremely cost efficient.

## Precision and repeatability

Precision (also referred to as repeatability by Cochrane [11]) refers to how consistently the test performs during repeated measurement, or the variations in the test itself. Terms that are often used with similar meaning are reliability and reproducibility, which refers to if the measurement can be reproduced with consistent results. In this discussion the precision and

repeatability of AiDA will be discussed in the terms of "short time repeatability" and "long term repeatability".

One of the most interesting features of the AiDA technique is that it consistently has been shown to perform measurements with high precision (e.g. < 7 µm for $r_{AiDA}$) even for repeated measurements on subjects at time intervals spanning over months or years.

## Short time repeatability of AiDA

As seen in Figure 17, repeated measurements on subjects have high short time repeatability, or precision. The standard deviation for repeated recovery measurements (Paper II) was determined to be within 0.002-0.008 which can be compared to the total range of $R$ for the group, which was 0-0.65 in that study. This means that the measurement uncertainty for measurements on subjects was 26-50 times smaller than the inter subject variability, even for a small group of young, healthy subjects.

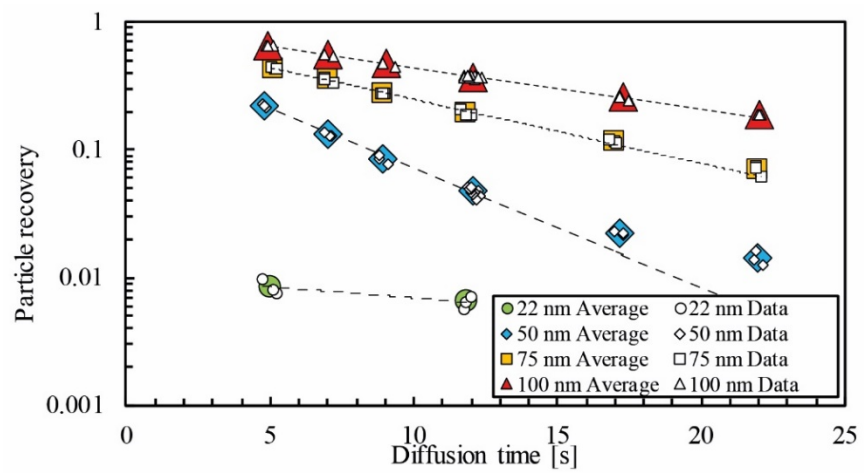


**Figure 17:**
The figure show data for particle recovery measurements on one subject in Paper II and particle sizes from 22 nm to 100 nm and comparison to the average values. As seen the short time repeatability, or precision, is high.

## Long-time repeatability of AiDA

Repeated measurements were performed on one subject (used as a bio control) during the measurements presented in Paper III. The measurements were performed with 50 nm particles and a 10 s breath-hold from a volumetric sample depth of 1800 mL at 18 occasions spread over one year. The result was determined to $R$ = 0.054 ± 0.003 (average ± 1 SD). Data for measurements with different breath holding times, with 50 nm particles and 1800

mL volumetric sample depth were also available for this subject for measurements performed 2013 - 2018, and can be seen in Figure 18:

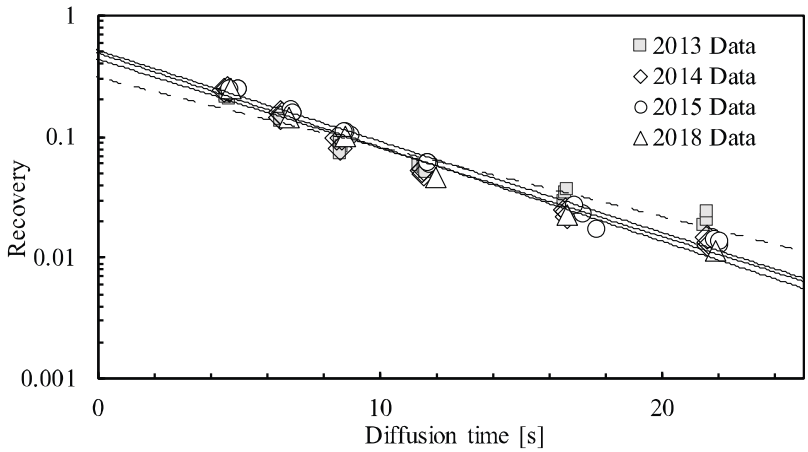


**Figure 18:**
Data for one subject, 50 nm particles and breath holding times measured from 2013 - 2018. The first measurement at 2013 shows elevated recovery values suspected to be caused by insufficient particle size selection, (some 100 nm particles are suspected to have passed the DMA, which would explain the observation, but cannot be confirmed at this point). Measurement 2014-2018 are in much better agreement and show the long time repeatability and precision of the AiDA technique.

Duplicate and triplicate measurements were performed on three subjects for a more comprehensive measurement program, including 48 measurements of recovery and determination of $R_0$ and $r_{AiDA}$ and presented in Paper IV. The measurements are shown in Figure 19. The measurements show that $R_0$ and $r_{AiDA}$ can also be measured with high precision. The $r_{AiDA}$ values between 1000-2500 mL varied within ±7 µm (2.4%) on average when comparing the repeated measurements for the three subjects (Paper IV).

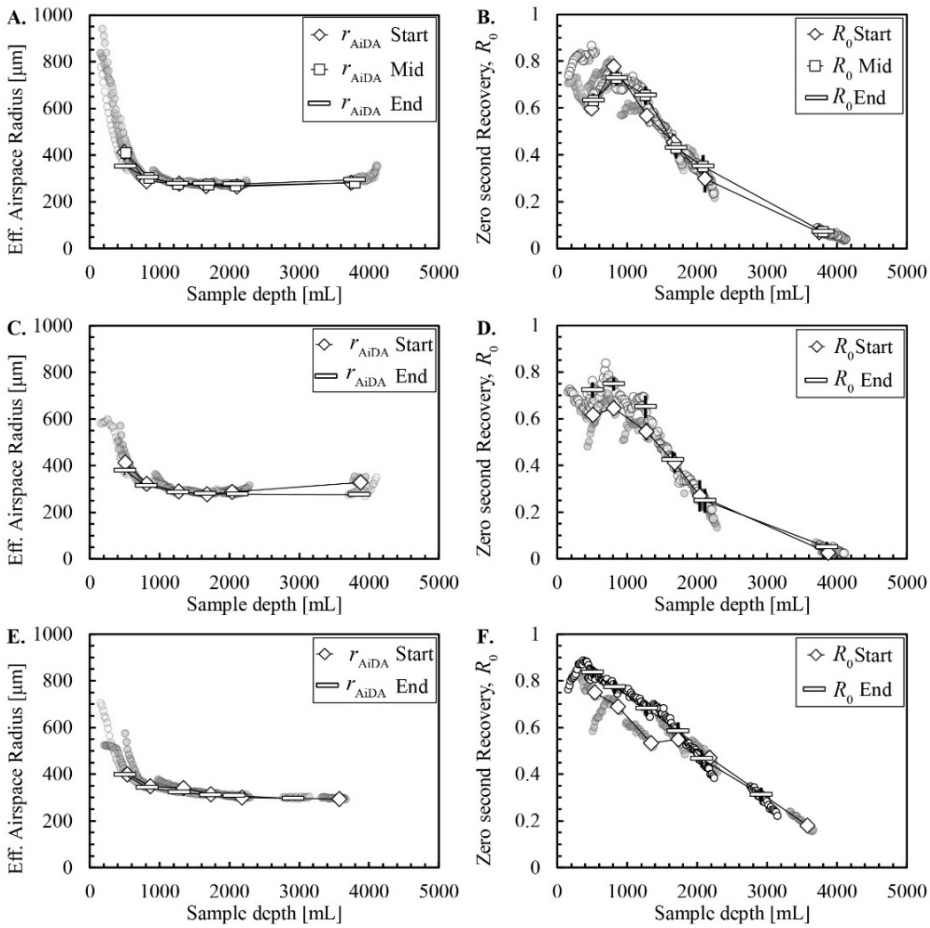


**Figure 19**
Repeated measurements on three subject, performed in intervals with a spread over several months (from Paper IV). A, B: Triplicate measurements on one male subject, 37 yr. at the first measurement, with the two later measurements at 10 and 18.5 months after the first measurement. B,C: Duplicate measurements on a male subject, 38 yr. at the first measurement, and 17 months between the measurements. E,F: Duplicate measurements on a male subject, 63 yr. at the first measurement, and 15 months between the measurements. As seen the repeatability is high, especially for sample depths > 1000 mL.

# Sensitivity and Specificity

Sensitivity refers to the ability of a test to give a positive finding when the subject who the test is performed on has the disease or abnormality under investigation [11]. Specificity is defined as the ability of the test to give a negative finding when the individual does not have the disease or abnormality under investigation [11]. As AiDA is not yet in clinical use it is not at this point possible to assess sensitivity or specificity in relation to clinical diagnosis, but some qualitative discussion based on the published results, and other available data, can be offered.

The only paper included in this thesis that publish AiDA data for subjects with respiratory disease is Paper III. In this study it is shown that particle deposition, reported as time normalized *DF* is significantly lower (p = 0.01-0.001) for COPD subjects than for healthy subjects. It is also shown that *DF* correlates with $D_{L,CO}$ for subjects with emphysema. This indicates that the AiDA technique have sensitivity for respiratory disease. It is also shown in Paper IV, in the related publications [48, 108] and in the preliminary data, that AiDA measurements correlate with subject characteristics and the results of lung function tests, MRI [108] and CT densitometry [14]. All of these factors indicate that AiDA measurements reflect lung properties, in agreement with the hypotheses in Paper I. Whether the technique is sensitive and precise enough to detect and quantify pathological changes to the lung, and at what stage, remains to be studied further.

It should be noted that the current analysis of AiDA measurements by calculating $R_0$ and $r_{\mathrm{AIDA}}$ is helpful for reasoning about the mechanisms involved, as it provides an intuitive description, but that there may be alternative ways of analysing the results that are more suited for diagnostic use.

For assessment of sensitivity and specificity to respiratory disease, the AiDA measurements may need to be adjusted for subject characteristics (age, sex, height, weight or VC) and compared with reference values. For instance, at present the measurements are performed at and reported for fixed volumetric sample depths for all subjects. For diagnostic use it may be more valid to normalize the results to %VC, as shown in Paper IV. This is especially true for $R_0$, which has been shown to depend strongly on volumetric sample depth (Figure 16, 19). Effective airspace radii are less sensitive given that they are assessed at sufficient volumetric sample depth. Normal reference values for healthy subjects remain to be established.

# Recommendations for AiDA measurements

The following section is based on practical experience of performing AiDA measurements, and analysis of AiDA data. The aim of this section is to highlight some factors that have been found to be crucial for the outcome of successful AiDA measurements. The section discusses the technique from the perspective of performing valid measurements of $r_{AiDA}$ and $R_0$ from one fixed volumetric sample depth according to the standard short measurement protocol described in the method section.

The measurements performed with the AiDA instrument are relative measurements, thus it is important that both the reference (the inhaled test aerosol number concentration and particle size) and the exhaled number concentration in the exhaled sample are determined in similar ways. It is also important that the residence time in the lung and the volumetric sample depth can be assessed accurately.

From experience, the most common cause of uncertainties and unsuccessful measurements are related the inhalation of the test aerosol, the collection of the exhaled sample and low particle concentrations in the exhaled sample. With exception for the problems discussed earlier related to the compliance for subjects with severe respiratory disease, the most common encountered problems regarding the breathing manoeuvres are slow and/or uneven inhalation of the test aerosol and fast and/or uneven exhalation of the sample. If the subject performs a slow or uneven inhalation of the test aerosol, uncertainties arise to when the test aerosol has reached the lung region of interest. It is also possible that the subject triggers the automatic valve system, and thus does not perform a full inhalation or test aerosol to TLC. Thus, the recommendation is to instruct the subject to inhale forcefully to total lung capacity. If the subject performs a fast exhalation, the limited time resolution of the pneumoptachograph/automatic sample valve may be insufficient to accurately control the volumetric sample depth from which the sample is collected, as discussed in the method section. If the subject performs an uneven exhalation this affects the determination of the residence time and volumetric position of the exhaled sample in the lung. An uneven exhalation may also introduce additional mixing of the sample. To avoid these problems, subjects can be instructed to exhale in an even, normal breath, like a sigh.

The problem with low particle concentrations in the exhaled sample is also critical to control for successful AiDA measurements. For healthy individuals and longer breath holds, exhaled particle levels can reach very low levels (<100 $cm^{-3}$). If the particle concentrations are too low, the particle counting statistics become uncertain and also minimal leaks at for instance the mouthpiece could influence data. As a rule of thumb, based on experience, exhaled particle concentrations < 100 $cm^{-3}$ should if possible be avoided. Possible ways of increasing the exhaled particle concentration includes increasing inhaled particle concentration, using shorter breath holding times and measure at lower volumetric sample depths.

Increasing the inhaled particle concentration is limited by both the technology for producing sufficiently monodisperse test aerosol and concerns for exposing subjects for unnecessary high amounts of particles. At particle levels $>5 \cdot 10^5$ $cm^{-3}$, coagulation may start to occur [26], which affects the monodispersity and stability of the aerosol. Normal recovery values for 50 nm particles are usually in the range of approximately 0.01-0.1. This, in combination with the recommendation to avoid particle concentrations < 100 $cm^{-3}$, sets a theoretical ideal inhaled concentration to around 10 000 $cm^{-3}$. Concentrations in the range 3000-8000 $cm^{-3}$ were used in the different studies, and mostly generated sufficient results with some exceptions. A possible effect of low exhaled particle concentration can be seen in Paper II, where recovery for 50 nm particles appear to be elevated for longer breath holding times (Figure 3 in Paper II,). A rule of thumb for successful measurements (based exclusively on experience) is to avoid measuring at conditions resulting in particle recovery values < 0.005. It should also be noted that production of monodisperse nano aerosol is technically challenging, thus increasing the particle concentration is not always an option.

The most convenient experimental parameter to change during AiDA measurements is the breath holding time. The consideration for this parameter is that the aerosol should reside long enough in the lung region of interest compared to the time it flows in and out of the lung, but not so long that the exhaled particle concentration reaches low concentrations as previously discussed. The analysis of the data obtained in the studies shows that the best results are obtained in the interval 5-10 s breath-holding times for 50 nm particles.

The volumetric sample depth was investigated in Paper IV. It was shown that the most consistent values for $r_{AiDA}$ were observed at volumetric sample depths between 1000 - 2500 mL. $R_0$ values were also most consistently assessed for sample depths > 1000 mL.

In Paper I, a suitable particle size range is estimated, based primarily on calculations, to 40-100 nm. Different particle sizes were examined in Paper II and III where 22 nm, 50 nm, 75 nm and 100 nm particles were used. As expected, close to 100 % deposition was observed for 22 nm particles even at short breath holding times. Particles < 30 nm are also known to deposit to a large extent in the upper airways due to their fast diffusion rate. Thus, this establishes a lower size range for the AiDA method. Particles larger than approximately > 300 nm are to increasing extent affected by other deposition mechanisms than diffusion and thus establish a likely upper limit. In one of the few studies comparing lung deposition of nanoparticles for healthy and diseased subjects [43] it was shown that deposition was decreased for COPD subjects for particle sizes < 100 nm, and slightly increased for larger particles during continuous breathing. As the deposition was almost the same for the subjects at 100 nm, it was initially assumed that particles < 100 nm was required for the diagnostic application of the AiDA technique. However, the particle size range for AiDA could be further optimized in the size range 50 - 100 nm. A rationale for this is that increased particle size entails slower diffusion rates, and this, combined with slightly increased breath holding times may increase the precision of the technique.

An important practical question for AiDA measurements is the number of measurements necessary for accurate determination of $R_0$ and $r_{AiDA}$, and the distribution of breath holding times. In Paper IV, measurements at 8 breath holding times were performed at each volumetric sample depth. To investigate the influence of the number and distribution of measurement points a sensitivity analysis was performed by systematically determine $R_0$ and $r_{AiDA}$ for all possible combinations of 8 – 3 of these measurements at each volumetric sample depth. Sensitivity analysis for one subject is shown in Figure 20:

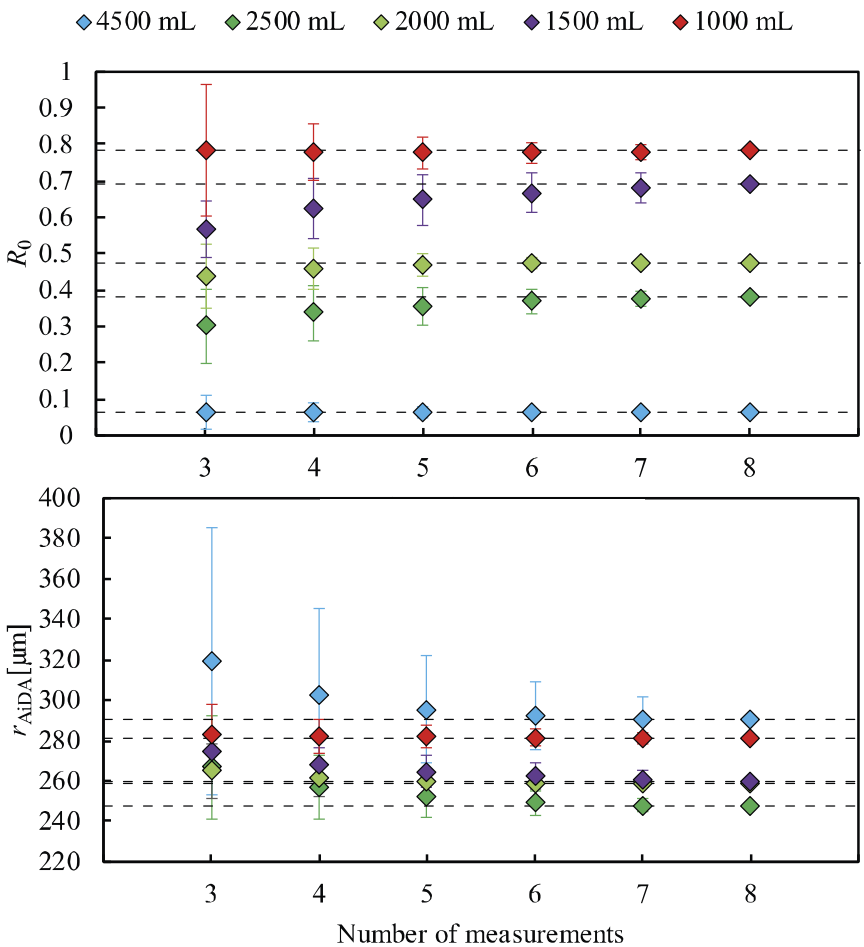


**Figure 20:**
Sensitivity analysis for 1000 – 4500 mL sample depths for one subject. Average and SD for $R_0$ (upper panel) and $r_{AiDA}$ (lower panel) calculated from all permutations of 3-8 breath holding times at each volumetric sample depth. Breath holding times varied between 5-20 s. The number of data points used are given on the horizontal axis. The baseline (fit for all 8 data points) is indicated as horizontal lines. It can be seen that the measurement uncertainty (the variation of the result approximated with the standard deviation) decrease with the number of measurements.

The analysis was performed by systematically combining 3 - 8 of the 8 measurements points with different breath holding time at each sample depth and perform an AiDA analysis for these data points. This resulted in an estimation of the uncertainty for determining $R_0$ and $r_{AiDA}$ as the average and standard deviation for measurements with different number of data points. It can be seen in Figure 20 that the uncertainty increase with less data points (toward the left side of the diagrams in Figure 20), and that 5-6 measurements seem to be sufficient for most combinations, while the use of 4 or less data points give increased uncertainty. Further analysis showed that even a small number of measurement points can give good fits if the distribution of the data points is sufficient. Choosing data points predominantly at shorter or at longer breath holding times, especially long breath holds, skewed the results. It can also be seen that while uncertainty decreases with sample depth for $R_0$, it increases with sample depth for $r_{AiDA}$. For $r_{\mathrm{AiDA}}$ this is an effect of low exhaled particle concentrations and poor counting statistics. The uncertainty also seems larger for $R_0$ at intermediate values (1500 – 2500 mL) and lower number of measurement points, which may be an effect of the strong effect on sample depth for this variable (see Figure 16, 19). Breath holds should be evenly distributed, and it can be suspected that long breath holds (>10 s) should best be avoided as they may introduce uncertainty from poor counting statistics. Thus, analysis of 4-6 measurements of recovery seems a likely minimum number of measurement points for acceptable measurement performance, and 1000 – 2000 mL an optimal sample depth range for most subjects. Suitable values for performing AiDA are summarized in Table 10.

**Table 10:**
Recommendations for standard AiDA measurements. The recommendations are based on the result in the articles and practical experience. The recommendations are given for standard measurements from a fixed sample depth of 1300 mL and with 50 nm particles.

| Experimental parameter | Recommendation | Comment |
|---|---|---|
| Inhalation | Forceful, even. | Important to instruct the subject to fill the lungs sufficiently. |
| Exhalation | Not forceful, even. | Instruct the subject to perform the exhalation “like a sigh”. |
| Inhaled particle concentration | 10 000 $cm^{-3}$ | > 3000 $cm^{-3}$ is probably sufficient for most situations. |
| Exhaled particle concentration | > 100 $cm^{-3}$ | If possible, determined by experimental parameters. |
| Lowest particle recovery | > 0.005 | Based on experience. |
| Breath holding time | 5-10 s | The breath hold should be at least as long as the dynamic phase of the measurement, but not long enough to cause too low exhaled particle concentration. |
| Volumetric sample depth | 1000 – 2000 mL | Lower sample depths usually less consistent. Low exhaled concentrations at deeper sample depths. |
| Particle size | 50 -100 nm | May be further optimized. |
| Number of measurements | > 4 | Depending on application. Optimization of the breath holding times may allow for sufficient accuracy with few measurements. |

# Summary and conclusion

## Summary

In summary the initially stated research objectives were addressed accordingly:

### 1: Development of AiDA theory

The relation between lung deposition of nanoparticles and lung anatomy was investigated. A model was constructed which can be used to derive an effective airspace radius in the distal lung from measurements of lung deposition of nanoparticles during a breath hold. The effective airspace radii, $r_{AiDA}$, are expressed as a root mean square distance to surfaces in the lung and reflect lung morphology. A secondary variable named the zero second recovery, $R_0$, is also derived from the model and conceptually accounts for particle losses during inhalation and exhalation of the test aerosol, although the theoretical background is somewhat more complicated. Both variables are likely to have clinical relevance, and compared with previously developed aerosol-based techniques, AiDA is expected to have several advantages. The advantages include more natural breathing patterns during measurements, an enhanced sensitivity for detection of enlarged airspaces, a simpler interpretation of the measurements as they can be expected to be almost exclusively due to diffusion, less deposited mass in the lung during measurements and an enhanced penetration of the test aerosol into damaged and poorly ventilated airspaces.

### 2: Development of AiDA instrumentation

An instrument was developed for measurements of lung deposition efficiency of inhaled nanoparticles. The instrument can be divided into three sub systems: A system for generating test aerosol, a system for administering the test aerosol in inhalation experiments and a system to analyse the particle concentrations in the inhaled and exhaled aerosol. Generation of well-defined test aerosol was accomplished in a continuous flow system by aerosolizing polystyrene latex nanospheres by means of an electrospray aerosol generator, followed by size selection with a differential mobility analyser and dilution with particle free air. This resulted in a continuous supply of highly monodisperse test aerosol. A system for inhalation of test aerosol and collection of exhaled samples from controlled volumetric sample depths after controlled residence time in the lung was constructed. The system was

characterized for particle losses, and a model was constructed for accounting for losses in the instrument during measurements. Measurements of particle concentration in the inhaled test aerosol and in exhaled samples was accomplished with a CPC. The produced instrument is capable of precise measurements of lung deposition of nanoparticles in a single breath. It is, to the best of our knowledge, currently the only instrument with this capability.

## 3: Evaluation of the AiDA method in health and disease

Clinical studies were performed on both healthy subjects and subjects with respiratory disease with the constructed AiDA instrument. To date AiDA measurements have been performed on more than 1000 subjects, although the data analysis is not yet completed for all data. This exceeds the number of subjects in all previous reported studies on measurements of lung deposition of nanoparticles. It was found that the instrument was capable of a measurement precision much higher than inter subject variability (26-50 times higher on a small group of healthy young subjects, Paper II). It was also found that the recovery measurements were stable over time (Paper II, IV) and in reasonable agreement with modelling results (Paper II).

The AiDA instrument was evaluated by measurements on subjects with COPD and it was found that particle deposition was significantly different between subjects with COPD and healthy subjects, (Paper III), and that *DF* correlated with the severity of emphysema, assessed with $D_{L,CO}$ (Pearson´s r =0.80 - 0.85, $p < 0.002$) and CT [14]. This indicates that measurements with the instrument reflects lung morphology, and especially changes to lung morphology due to emphysema.

The instrument was evaluated by determining $r_{AiDA}$ and $R_0$ at volumetric sample depths ranging from close to anatomical dead space to close to VC for healthy subjects. It was found that $r_{AiDA}$ followed the general pattern of lung models based on histological measurements, but as expected the root mean square distances measured with AiDA were larger than the geometrical means given for these models. Also $R_0$ followed expected trends. Repeated measurements on three individuals performed with 15 - 18 months apart (Paper IV) showed that the precision of $r_{AiDA}$ was on average within ± 7 µm, or 2.4 % of the $r_{AiDA}$ values.

In the different studies, it was also found that AiDA measurements reported both as total particle deposition and as $r_{AiDA}$ and $R_0$ correlated with subject characteristics such as age, length, weight and with the results of several clinical techniques. This further supports that AiDA measurements reflect lung morphology and physiology. Importantly, $r_{AiDA}$ and $R_0$ does not correlate strongly and therefore may provide information of different lung properties. The value of AiDA as a future diagnostic technique remains to be investigated further.

# Conclusion

The Airspace Dimension Assessment method has potential to derive information about the lung not easily obtainable by other means. A theoretical framework was developed for the method, and an instrument was constructed. The instrument is capable of precise and accurate measurements of lung deposition of nanoparticles in a single breath.

By measurements of aerosol with different residence time in the lungs, an effective airspace radius in the distal lung, $r_{AiDA}$, and a zero second recovery value, $R_0$, can be determined. Effective airspace radii, $r_{AiDA}$, reflect the geometry of the human lung, but should not be understood as a measurement of a bronchiole calibre or alveolar radius, but as a root mean square of all distances between surfaces at the volumetric sample depth it was measured. The zero second recovery, $R_0$, can conceptually be understood as reflecting particle losses during the dynamic part of AiDA measurements, and therefore can be assumed to reflect properties of mainly the small conducting airways, although this variable remains to be studied further. Importantly, effective airspace radii and zero second recovery do not correlate, and can thus be expected to provide independent information about the lungs.

The measurements are simple both to administer and to perform for most subjects. Lung deposition measurements with the AiDA instrument was shown to differ between heathy subjects and subjects with respiratory disease, which indicates that the technique may provide information about abnormalities in the lungs.

AiDA measurements have been performed on a large number of subjects (>1000 individuals), and it has been shown that the method correlates with subject characteristics and lung function tests, including diffusing capacity for carbon monoxide and forced oscillation technique. Results from a comprehensive study with AiDA on a group of healthy subjects suggest that the method can be used to chart airspace distances in large parts of the gas exchange region of the lungs. This region of the lungs is not easily accessible to measure by other means. A possible future application for the AiDA technique may be early diagnosis of chronic obstructive pulmonary disease. However, the sensitivity and specificity for the AiDA technique for different diseases remain to be further investigated.

# Outlook

This thesis hopefully provides a basic understanding of the AiDA technique, although answered questions have also resulted in new ones. The research is in progress and several more studies are ongoing or planned.

## Development of AiDA technology

At the time of writing of this thesis, the second generation of the AiDA instrument is in the final stages of production. The design of the new prototype is to a large extent a product of a master thesis in industrial design [142] and incorporates much of the experiences from working with the first prototype of the AiDA instrument described in Paper II. The new design is hopefully more suited for practical clinical use, with the ergonomics of both the operator and the subject in mind.

Even though the new prototype was redesigned it is still based on the same technology as the original setup, including some expensive and high tech components i. e. the electrospray aerosol generator and the condensation particle counter. Some efforts have been made to replace these components with less expensive alternatives and should be pursued in the future. Further development of the AiDA measurement procedure may also be possible, such as further optimization of the particle size used in the measurements and improved criteria for data quality assessment.

## Future research needs and planned work

AiDA measurements seem to provide information about the lung not easily obtainable by other means, but the value of the technique as a diagnostic tool remains to be studied further. Models for reference values, adjusted for subject characteristics such as age, length, weight and gender must be developed for normal subjects. Also more data for subjects with respiratory disease is needed to evaluate sensitivity and specificity for the method with regard to respiratory disease.

A better understanding of the zero second recovery, including theoretical models, is needed for analysis and interpretation of this variable. The zero second recovery may provide important information about the small conducting airways, (which is believed to be where the first changes in COPD manifests), or the convective airflows in the lung, but must be better understood. The zero second recovery could be investigated in studies with more controlled breathing patterns, including systematic variations of respiratory flow rate and studies including different particle sizes. Developments of AiDA "bolus" techniques and studies with physical models with known geometry such as packed beds or diffusion batteries are also possible alternatives.

The SCAPIS data set is to date the largest data material with measurements of lung deposition of nanoparticles in human subjects, alone exceeding the total number of subjects in all previous studies combined. In this data set the subjects are also very well characterized by questionnaires, lung function tests, computed tomography of the lungs and Impulse Oscillometry. The data set remains to be extensively studied and hopefully provides a source for new discoveries and deeper understanding of AiDA.

In addition to the SCAPIS study, a specially interesting study that investigates AiDA for phenotyping of COPD is in progress [143]. In this study subjects suffering from alfa-1-antitrypsin deficiency are compared to subjects with "normal" COPD. Alfa-1-antitrypsin deficiency is a genetic disorder that causes severe but "pure" panacinar emphysema, but normally no bronchiolitis. Normal COPD patients usually have both centriacinar emphysema and bronchiolitis. The hypothesis is that AiDA will detect enlarged airspaces on both groups, but normal zero second recovery for the Alfa-1-antitrypsin patients compared to lower values for the normal COPD subjects caused by bronchiolitis. Preliminary results indicate that this is the case [143].

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# AiDA - a new diagnostic method

Breathing is essential for our existence. We can survive a few weeks without food and a few days without water, but only a few minutes without air. Even a slight impairment of this critical function can have a large impact on our quality of life. Small changes and abnormalities in the distal airspaces of the lungs is often the first stage of serious respiratory disease, however few techniques are available which are able to detect such changes, especially at an early stage. This thesis describes a putative new method that can be used to detect, and perhaps even quantify, small alterations in the lungs by measurements of lung deposition of inhaled nanoparticles.

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