Immunogold labelling of allergens at fine dust filter particles for SEM investigations

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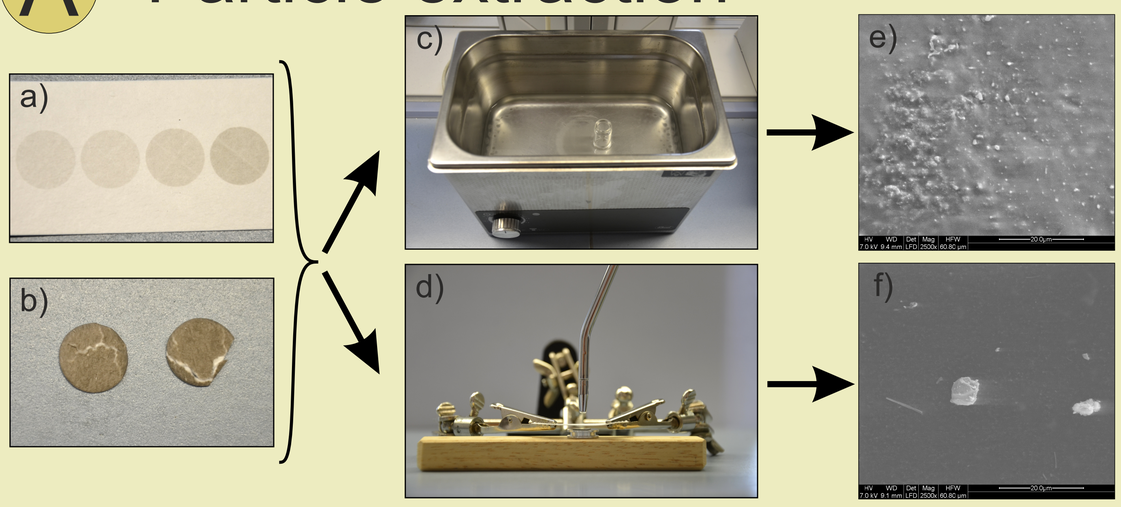
Introduction

In the last decades the number of people with allergies against at least one of the major allergens like birch, grass, ragweed pollen or domestic cat steady increases [1].There are various investigation methods available with which new insights were gained regarding the allergy potential, the concentration or position of specific allergens at various sample types. A more sophisticated method is immunogold electron microscopy, which enables to locate specific proteins which are responsible for an allergic reaction. Because of their high spatial accuracy and the used small gold particle size (approximately 10 – 60 nm), this method is typically applied for transmission electron microscope (TEM) investigation [2]. The investigations presented were performed to obtain information about the presence of specific allergens at particles deposited at fine dust filters. Because of the sample size they have to be examined in a scanning electron microscope (SEM). Unfortunately, several examinations have shown that typical TEM labelling protocols are not directly transferable for SEM investigations. Therefore the immunogold labelling protocol was adopted to enable labelling at the samples under investigation. The so developed strategy involves the extraction of the particles (A) wich can subsequently be labelled by the obtained protocol (B).

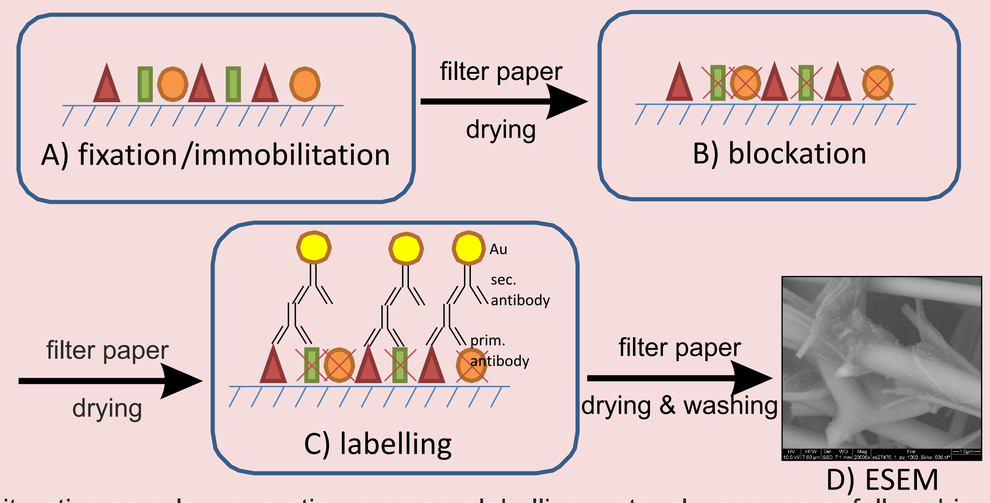
Methodology and results

A Particle extraction



Particles were collected on the bases of either a) hour or b) diurnal mean on fine dust filters. To enable the immunogold labelling investigation has been shown, that the particles have to be extracted from the filters. Therefore two different strategies were found: For the first particles were extracted by c) immerging the filter into liquid, ultrasonic it for about 5 min and subsequently evaporate the liquid on a carbon tape, see e). The second strategy d) uses compressed air to directly extract particles from the upside down clamped filter onto the tape, see f). First results show that the extraction efficiency is higher for the ultrasonic strategy, but a possible germination of possibly deposited pollen by contact with liquids can be neglected for the second strategy.

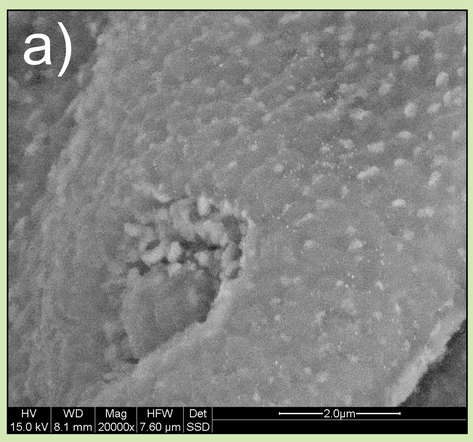
B Immunogold labelling protocol



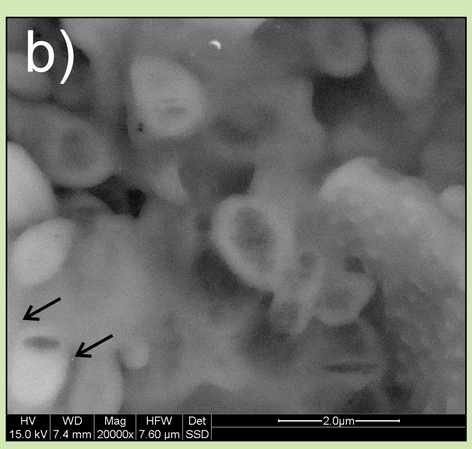
By an iterative sample preparation process a labelling protocol was successfully achieved. At first the prepared sample underwent an fixation/immobilisation step by the usage of bovine serum albumin (BSA), to fix the proteins under investigation at its initial position. Subsequently, a blocking step with glutaraldehyde (GA) was performed to suppress a unwanted labelling of background. The labelling of the allergens of interest is performed by using appropriate monoclonal primary antibodies, which are already covalent bound to the secondary antibody and thus gold-nanoparticle. After final washing steps this samples then can by directly investigated in the environmental SEM (ESEM).

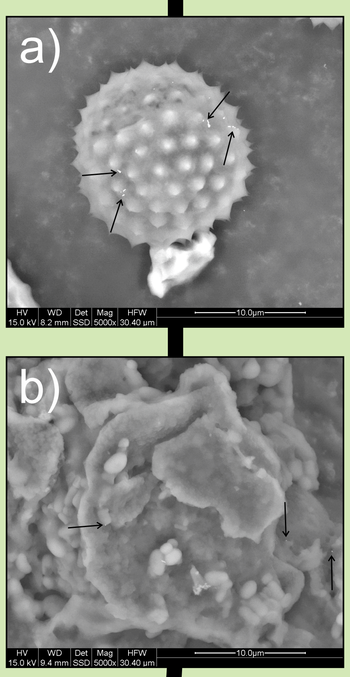
C Results, discussion and outlook

Initially the obtained labelling protocol was tested at cryo-milled birch and grass pollen for identifying the presence of the major grass pollen allergen Phl p5. As shown in Img. 1 a high signal (immunogold concentration) can be found at the cryo-milled grass pollen at a), while only a small signal was detected at the appropriate birch pollen at b), representing the background labelling and specificity of the used primary antibody.

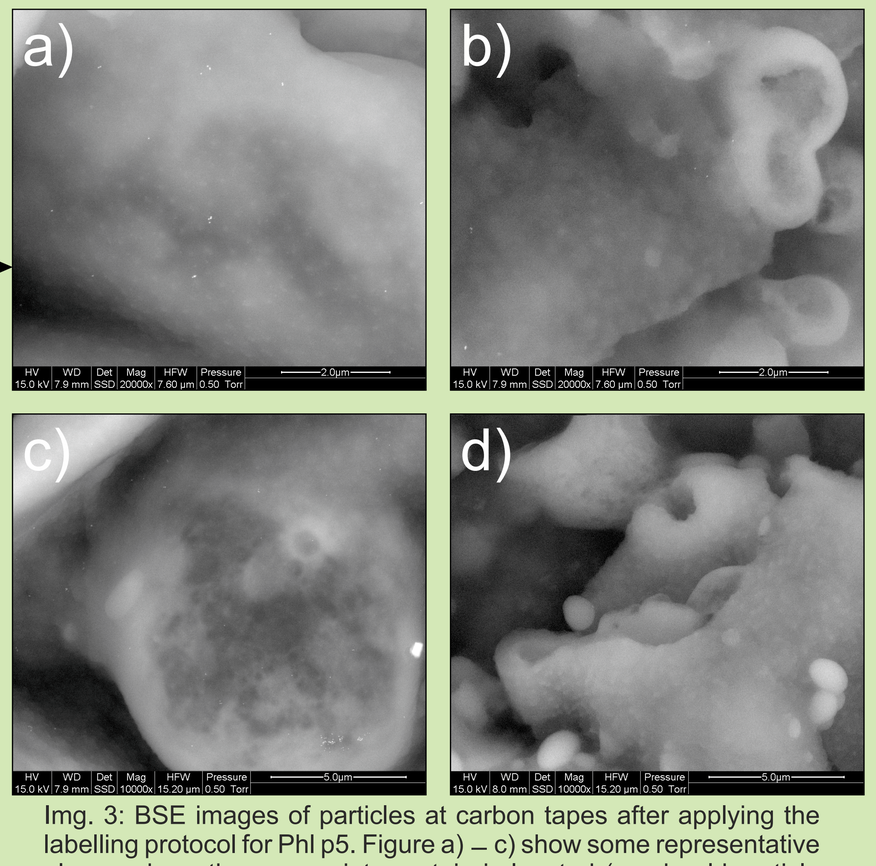


Img. 1: Backscatter electron (BSE) images show the result of testing the obtained immunogold labelling protocol for the major grass pollen allergen Phl p5 at a) cryo-milled grass pollen as positive control and b) at cryo-milled birch pollen as negative control. Used gold particle size: 20 nm.





Img. 2: Adapted immunogold labelling protocol for ragweed allergen Amb a1 tested at a) ragweed pollen after germination with rainwater (positive control) and b) at cryo-milled grass and birch pollen (negative control). The used gold-particle size was 60 nm.



Img. 3: BSE images of particles at carbon tapes after applying the labelling protocol for Phl p5. Figure a) – c) show some representative places where the appropriate protein is located (used gold-particle size 20 nm). Figure d) show the control specimen, where cryo-milled birch pollen was used.

✓ successful testing of obtained labelling protocol for
- grass pollen allergen Phl p5
- ragweed pollen allergen Amb a1

→ further improvement of particle extraction to improve statistics
→ expand sample preparation technique to analyse further allergens or pathogenic germs

Acknowledgements and references

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