The EU FP7 NanoDefine Project

Development of an integrated approach based on validated and standard-
ized methods to support the implementation of the EC recommendation
for a definition of nanomaterial

Summer school providing practical train-ing in the developed methods

NanoDefine Technical Report D8.15

W. Beek and H. Marvin

The NanoDefine Consortium 2017

NanoDefine in a nutshell

The EU FP7 NanoDefine project was launched in November 2013 and will run until October 2017. The project is dedicated to support the implementation of the EU Recommendation on the Definition of Nanomaterial by the provision of the required analytical tools and respective guidance. Main goal is to develop a novel tiered approach consisting of (i) rapid and cost-efficient screening methods and (ii) confirmatory measurement methods. The "NanoDefiner" eTool will guide potential end-users, such as concerned industries and regulatory bodies as well as enforcement and contract laboratories, to reliably classify if a material is nano or not. To achieve this objective, a comprehensive inter-laboratory evaluation of the performance of current characterisation techniques, instruments and software is performed. Instruments, software and methods are further developed. Their capacity to reliably measure the size of particulates in the size range 1-100 nm and above (according to the EU definition) is validated. Technical reports on project results are published to reach out to relevant stakeholders, such as policy makers, regulators, industries and the wider scientific community, to present and discuss our goals and results, to ensure a continuous exchange of views, needs and experiences obtained from different fields of expertise and application, and to finally integrate the resulting feedback into our ongoing work on the size-related classification of nanomaterials.

Bibliographic data

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1   Summary

The NanoDefine Summer School on *training in the developed methods* took place in Wageningen, The Netherlands, on June 21-23, 2017, and was organized by beneficiary RIKILT. The course was advertised within the NanoDefine consortium, at the NanoDefine beneficiaries, on the NanoDefine public website, in the NSC newsletter, and by a direct email announcement to > 100 stakeholders. Since, this event was a hands-on training, maximal 20 persons could attend. In total, 18 (1 NanoDefine partner and 17 external) researchers from different countries and nationalities participated in this course. The course was given by lectures and trainers of the NanoDefine consortium (beneficiaries JRC-Ispra, EWAG, CODA-CERVA and RIKILT) The course provided training on the tiered measurement approach, the application of the Particle sizer software for TEM/SEM pictures, and the NanoDefiner eTool which have been developed by NanoDefine for the classification of nanomaterials. The participants gained practical experience in i) sample preparation for sp-CPMS and SEM/TEM microscopy, data processing of sp-ICP/MS data and SEM/TEM pictures, and the function and use of the NanoDefiner eTool.

2 Introduction

The concept of the European FP7 project NanoDefine is the development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation of the for a definition of nanomaterial. The major outcome of the project is the establishment of an integrated tiered approach based on validated rapid screening methods (tier 1) and validated confirmatory methods (tier 2). With the developed NanoDefiner eTool and user manual, end-users will be guided to implement the developed methodology.

The objectives of the NanoDefine Summer School was to provide a training on (i) the tiered measurement approach and NanoDefine's eTool (developed by NanoDefine) for classification of nanomaterials, (ii) practical experience in the use of the NanoDefiner eTool, from handling of raw data, such as EM pictures or spICPMS output, to implantation into the NanoDefiner, and processing of spICPMS data (iii) practical training on spICPMS and Electronic Microscopy (EM). The participants were given practical experiences in i) sample preparation for spICPMS and SEM/TEM, data processing of spICPMS data and SEM/TEM pictures and the Particle sizer software, and the function and use of the NanoDefiner eTool.

The course was organized by beneficiary RIKILT in close cooperation with the NanoDefine partners JRC-Ispra, EAWAG, FHDO and CODA-CERVA. The training was held at the facilities of RIKILT which included lecture rooms, and two different laboratories for spICPMS and SEM/TEM. The software for spICPMS, Particle sizer, and the Nanodefiner eTool, were provided free of charge to the participants and installed on their own computer.

The course was advertised within the NanoDefine consortium, at the NanoDefine beneficiaries, on the NanoDefine public website, in the NSC newsletter and by a direct email announcement to > 100 stakeholders Participation

Due to the fact that hands-on training was provided, the maximal capacity was limited 20 persons. In total, 18 (1 NanoDefine partner and 17 external) researchers from 9 different countries participated in this course. To ensure knowledge transfer at expected level, only participants having experience with either spICPMS or EM were granted the participation. They represented testing laboratories, academia and research institutes originating from Czech Republic, Italy, Netherlands, Norway, UK, Brazil, Germany, Austria and Spain.

A photo of the participants (and lecturers/trainers) is provided in Figure 1.

Figure 1: Course participants and trainers

3 Course program

The course was composed of lecturers (on day 1, 2 and 3) on nanomaterials, tier 1 and tier 2 methods, the NanoDefiner eTool, introduction to spICPMS and Electron Microscopy Analysis, and the use of data-processing (spICPMS and SEM/TEM analysis). The hands-on training consisted of: i) practical training (laptop) in the NanoDefiner eTool and laboratory work (preparing the samples for spICPMS and TEM. An overview of the detailed program is given in Annex 2.

The original concept of the program was to split (on day 2 afternoon – the lab training) the practical training in two different groups, a sp-ICPMS group and an Electron Microscopy group. As a consequence everybody was trained in one technique and not in both. At request of the course participants, this concept has been abandoned and the program was modified into training on both techniques for all. The detailed course program of day 2 is given in Annex 2.

All participants of the Summer School received a course book consisting of info of the NanoDefine project, prints (handouts) of all presentations and scientific publications. The presentations were provided electronically (as pdf) to all course participants (printed and on memory stick) and will be made available on the website of the NanoDefine project (www.NanoDefine.eu).

Photos illustrating actively participating in lectures and social get-together dinner are provided in Figure 2.

Figure 2: Course photos

4   Evaluation

At the end of the training course, the participants were asked to complete a short questionnaire and to comment on different aspects of the NanoDefine Summer School. 17 out of 18 of completed questionnaires were recovered. The feedback received was very positive in all aspects (facilities, programme, trainers, etc.).

Evaluation of the questionnaires: Generally the course was very useful and motivating, the speaker's presentations were enjoyable and of high quality, the organization and support were efficient and provided facilities were very good. The following aspects were experienced as very useful: (i) general introductions and background information, (ii) the introduction to spICPMS and EM-microscopy, (iii) the hands-on-training on spICPMS and EM-microscopy, (iv) the training in using the NanoDefiner eTool, (v) the provided information. The course provided information as expected; application of obtained information is transferable in the participant professional activities. The provided training materials were very useful.

Some suggestions/comments given - to improve the course - were also collected from the attendees:

- *It was too much, too fast. Some more days would be helpful.*
- *Add a troubleshooting item*
- *Techniques explanation was helpful/good, even for experienced people.*
- *Sample preparation: more time in the lab, at least one day*
- *No objections to have different techniques*
- *SOPs should be included in the NanoDefiner eTool*
- *Contamination of NanoDefiner eTool: will be worked on*
- *Electron Microscopy – software for automatization as running at EAWAG will be free available*
- *Facilities were good.*

5   Conclusions

The Summer School was highly successful and very much appreciated by both the course participants. During the closing event, all participants received a Certificate of Training from Hans Marvin, coordinator of the NanoDefine project.

6   References

NanoDefine Grant Agreement number 604347, Annex I 'Description of Work'.

Appendix 1

NanoDefine Summer School
(organised by EC FP7 NanoDefine project partner)

Date: 21 – 23 June 2017
The concept of the European FP7 project NanoDefine is: Development of an integrated approach based on validated and standardized methods to support the implementation of the EC recommendation of for a definition of nanomaterial.
The major outcome of the project is the establishment of an integrated tiered approach based on validated rapid screening methods (tier 1) and validated confirmatory methods (tier 2). With the developed NanoDefiner eTool and user manual, end-users will be guided to implement the developed methodology.

SCOPE OF THE SUMMER SCHOOL
The Summer School will provide (i) training on the tiered approach and NanoDefine's eTool (developed by NanoDefine) for classification of nanomaterials, (ii) practical experience and use of the NanoDefiner eTool, from handling of raw data, such as EM pictures or sp-ICPMS output, to implementation into the NanoDefiner, and processing of sp-ICPMS data, (iii) practical training on sp-ICPMS and Electronic Microscopy (EM).

WHAT SHOULD BE EXPECTED FROM THE SUMMER SCHOOL
After attending the Summer School, participants will understand the concept of the NanoDefiner eTool, in connection with methods of analysis, such as spICPMS and Electron Microscopy, used.

WORKSHOP ORGANISER AND CONTACT INFORMATION
RIKILT Wageningen University & Research
P.O. Box 230, 6700 AE Wageningen, The Netherlands

Contact:
NanoDefine's Project secretary, Wim Beek, Email: Wim.Beek@wur.nl

REGISTRATION AND FEES
To register for the Summer School, please complete the Registration Form and email it to the address: wim.beek@wur.nl

The registration will be closed on April 15, 2017.

Please, note that while the participation to the Summer School is free, participants will be responsible for their travel, accommodation costs and own meals. There are only limited numbers of places available, and we may not be able to customize your preferences. Together with your registration, please submit also the enclosed survey. The completed survey is compulsory for your registration. If the survey is missing we cannot consider your registration.
You will be informed by email whether you are selected or not to attend the Summer School by April 30, 2017.
Only those candidates selected for the Summer School will receive further practical information regarding transport and accommodation.

Appendix 2

NanoDefine Summer School
Program

Date: Week 25: **21-23 June 2017**

Location: RIKILT Wageningen University & Research
          Wageningen Campus, Akkermaalsbos 2, (Building 123)
          6708 WB Wageningen, The Netherlands

Objectives

1) The Summer School will provide training to NanoDefine partners and external (young and experienced) researchers on the tiered measurement approach, the application of the Particle sizer software for TEM/ SEM pictures, and NanoDefiner eTool which have been developed by NanoDefine for the classification of nanomaterials.
2) The participants will gain practical experience in i) sample preparation for sp-ICPMS and SEM/TEM, data processing of sp-ICPMS and SEM/TEM pictures, the function and use of the NanoDefiner eTool and the Particle sized software,

Programme

Day 1:   **Wednesday 21 June 2017**
Time: 13.00-17.00
Location: RIKILT, room 0016-0017

12:00 – 13:00 Lunch at RIKILT

13:00

   Welcome and tour the table (who is who)

13:30

   Introduction to NanoDefine and to classification of nanomaterials for regulatory actions
   <u>Hans Marvin (RIKILT).</u>

14:00

   Introduction to tier 1 and tier 2 methods (pro and cons)
   <u>Hans Marvin (RIKILT) / Hubert Rauscher (JRC-Ispra)</u>

14:30

> Introduction to NanoDefiner eTool
>
> Hubert Rauscher (JRC-Ispra) / Christoph Friedrich (FHDO)

15:00 Coffee/ tea

15:15

> Demonstration of the NanoDefiner eTool
>
> Hubert Rauscher (JRC-Ispra) / Christoph Friedrich (FHDO)

End at 17:00

Dinner: 19.00 at   **Diels Restobar**
                       Vijzelstraat 2
                       6701 DC Wageningen
                       +31 317 422 694
                       www.dielsrestobar.nl

Day 2:   **Thursday 22 June 2017**
Location: RIKILT, room 0016-0017 and Lab; RADIX, room EM-lab/room W06
Time: 09:00 – 17:30

09:00

> Introduction to sp-ICPMS (group 1 in room 0016)
>
> Anna Undas (RIKILT) / Ruud Peters (RIKILT)
>
> Introduction to EM-microscopy (group 2 in room 0017)
>
> Evelyn Verleysen (CODA-CERVA) / Ralf Kaegi (EAWAG)

10:00

> Introduction to data processing spICPMS (group 1 in room 0016)
>
> Anna Undas (RIKILT) / Ruud Peters (RIKILT)
>
> Physical characterization of NM by EM in a regulatory framework (group 2 in room 0017)
>
> Evelyn Verleysen (CODA-CERVA) / Ralf Kaegi (EAWAG)

11:00

> Introduction to sample preparation (dispersing, enzymatic and chemical digestion) (group 1 in 0016) Ruud Peters (RIKILT)

Introduction to sample preparation EM (group 2 in 0017) Evelyn Verleysen (CODA-CERVA) / Ralf Kaegi (EAWAG)

Lunch 12:00 – 13:30

13:30 – 15:30
SEM/TEM-image analysis using the Particle Sizer (group 2a room EM lab)
Evelyn Verleysen (CODA-CERVA) or Ralf Kaegi (EAWAG)

15:30 – 17:30
EM: Sample prep / analysis (group 2a room EM lab)
Evelyn Verleysen (CODA-CERVA) or Ralf Kaegi (EAWAG)

13:30 – 15:30
EM: Sample prep / analysis (group 2b room EM lab)
Evelyn Verleysen (CODA-CERVA) or Ralf Kaegi (EAWAG)

15:30 – 17:30
SEM/TEM -image analysis using the Particle Sizer (group 2b room EM lab)
Evelyn Verleysen (CODA-CERVA) or Ralf Kaegi (EAWAG)

13:30 – 15:30
Data processing sp-ICPMS (group 1a room 0016)
Anna Undas (RIKILT) / Ruud Peters (RIKILT)

15:30 – 17:30
Sample prep/analysis sp-ICPMS (group 1a RIKILT lab)
Anna Undas (RIKILT) / Ruud Peters (RIKILT)

13:30 – 15:30
Sample prep/analysis (sp-ICPMS (group 1b RIKILT lab)
Anna Undas (RIKILT) / Ruud Peters (RIKILT)

15:30 – 17:30
Data processing (sp-ICPMS (group 1b room 0016)
Anna Undas (RIKILT) / Ruud Peters (RIKILT)

End at 17:30

Day 3:	**Friday 23 June 2017**
Time:	09:00 – 14:30
Location:	RIKILT, room 0016-0017

09:00

Practical training in using the NanoDefiner
- how it works
  Hubert Rauscher (JRC-Ispra)/ Christoph Friedrich (FHDO)

Practical training in classifying tested samples with the NanoDefiner
- comparison sp-ICPMS and SEM (from samples prepared on day 2)
  Hubert Rauscher (JRC-Ispra) / Christoph Friedrich (FHDO)

12:00 – 13:00 Lunch

13:00

Final discussion and evaluation
Ruud Peters (RIKILT) / Hans Marvin (RIKILT).

14:00

Handover of certificate of attendance
Hans Marvin (RIKILT).

14:30 Closing

(changed program day 2)

NanoDefine Summer School
Program

Date:	Week 25: **21-23 June 2017**
Location:	RIKILT Wageningen University & Research
	Wageningen Campus, Akkermaalsbos 2, (Building 123)
	6708 WB Wageningen, The Netherlands

Day 2: **Thursday 22 June 2017**
Location: RIKILT, room 0016-0017 and Lab; RADIX, room EM-lab/room W06
Time: 09:00 – 17:30

09:00

Introduction to sp-ICPMS (group 1 and 2 in room 0016-0017)
Anna Undas (RIKILT) / Ruud Peters (RIKILT)

Introduction to data processing spICPMS (group 1 and 2 in room 0016-0017)
Anna Undas (RIKILT) / Ruud Peters (RIKILT)

Introduction to sample preparation (dispersing, enzymatic and chemical digestion) (group 1 in 0016) Ruud Peters (RIKILT)

10:45 Introduction to EM (group 1 and 2 in 0016-0017)
Evelyn Verleysen (CODA-CERVA) / Ralf Kaegi (EAWAG)

Physical characterization of NM by EM in a regulatory framework
(group 1 and 2 in room 0016-0017)
Evelyn Verleysen (CODA-CERVA) / Ralf Kaegi (EAWAG)

Lunch 12:30 – 13:30

13:30 – 15:30
SEM/TEM-image analysis using the Particle Sizer (group 2a room EM lab)
Evelyn Verleysen (CODA-CERVA) or Ralf Kaegi (EAWAG)

15:30 – 17:30
EM: Sample prep / analysis (group 2a room EM lab)
Evelyn Verleysen (CODA-CERVA) or Ralf Kaegi (EAWAG)

13:30 – 15:30
EM: Sample prep / analysis (group 2b room EM lab)
Evelyn Verleysen (CODA-CERVA) or Ralf Kaegi (EAWAG)

15:30 – 17:30

    SEM/TEM -image analysis using the Particle Sizer (group 2b room EM lab)
    Evelyn Verleysen (CODA-CERVA) or Ralf Kaegi (EAWAG)

13:30 – 15:30

    Data processing sp-ICPMS (group 1a room 0016)
    Anna Undas (RIKILT) / Ruud Peters (RIKILT)

15:30 – 17:30

    Sample prep/analysis sp-ICPMS (group 1a RIKILT lab)
    Anna Undas (RIKILT) / Ruud Peters (RIKILT)

13:30 – 15:30

    Sample prep/analysis (sp-ICPMS (group 1b RIKILT lab)
    Anna Undas (RIKILT) / Ruud Peters (RIKILT)

15:30 – 17:30

    Data processing (sp-ICPMS (group 1b room 0016)
    Anna Undas (RIKILT) / Ruud Peters (RIKILT)

    End at 17:30

Appendix 3

Participants

First name	Last name	Affiliation
Karel	Lach	Institute of Public Health Ostrava, Czech republic
Šárka	Bártová	Public Health Institute Ostrava, Czech republic
Leonardo	Bellarosa	Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Italy
Anastasia	Georgantzopoulou	Norwegian Institute for Water Research, Norway
ALESSANDRO	SANTATO	SOILCARES, Wageningen, Netherlands
Nathaniel	Clark	Plymouth University, UK
Ilaria	Zanoni	University of Trieste and ISTEC-CNR of Faenza, Italy
Ana Isabel	Carrazco Quevedo	University of Birmingham, UK
Monique	Mendonça	University of Campinas, Brazil
Hana	Caloudova	University of Veterinary and Pharmaceutical Sciences Brno, Czech republic
Pirutchada	Musigapong	University of Birmingham, UK
Valerio	Funari	University of Bologna, Italy
Petra	FIALA	TU Dresden
Lodewijk	Steendam	Netherlands Food and Consumer Product Safety Authority
Dagmar	Koller	University of Cambridge, School of Biology and MRC-EWL
Dan	Hodoroaba	Federal Institute for Materials Research and Testing (BAM)
Sara	Taghavi	University of polytechnic of Catalonia (UPC), Spain University
Giulio	Cotogno	JRC-Ispra

Lecturers

Hubert	Rauscher	JRC-Ispra
Christoph	Friedrich	FHDO
Eveline	Verleysen	CODA-CERVA
Ralf Kaegi	Kaegi	EAWAG
Ruud	Peters	RIKILT
Anna	Undas	RIKILT
Hans	Marvin	RIKILT
Wim	Beek	RIKILT