The EU FP7 NanoDefine Project

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

Development of a cost-effective FFF-separation system for nanoparticle analysis

NanoDefine Technical Report D5.6

Ulrich Rösch

The NanoDefine Consortium 2017

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under Grant Agreement n° 604347

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:

NanoDefine Technical Report D5.6

Report title: Development of a cost-effective FFF-separation system for nanoparticle analysis

Author: Rösch U.[1]

Affiliation: Superon GmbH, DE-56307 Dernbach, Germany

Publication date: 25/10/2017

Publisher: The NanoDefine Consortium

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Place of publication: Wageningen, The Netherlands

URL: http://www.nanodefine.eu/index.php/downloads/public-deliverables

Contact: coordinator@nanodefine.eu, www.nanodefine.eu

[1] Corresponding author: ulrich.rosch@superon.eu

The NanoDefine project has received funding from the European Union's Seventh Programme for research, technological development and demonstration under grant agreement No 604347

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1 Abbreviations and acronyms

RC.Net	Rapid Control (dot) Net
ICF	Instrument Control Framework
FFF	Field Flow Fractionation
QELS	Quasi Elastic Light Scattering

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2 Summary

The development of a more cost-effective FFF solution was split into a hardware and software part.

On the hardware side we have achieved to most desirable solution with significantly optimized technical characteristics at lower prices and substantively reduced service costs. The customers can decide themselves which peripheral hardware fits best into their budget and applies the needs of the application. The connective centerpiece between hardware and software is the Rapid Control Interface built-in our new data acquisition software VOYAGER CDS®. This allows the use of every RC.Net compatible HPLC module and the FFF the same time. Because VOYAGER CDS® is made according the moderate demands of FFF, we were able to create a small software solution with very interesting price compared with the well-known standard platforms. VOYAGER CDS® as pure data acquisition software works seamlessly together with our new data processing solution SCOUT DPS®. This program guides the customers through all steps of method development by its easy-to-use interface, chromatogram prediction in real time and saving of the optimized method, ready to be instantly executed by the hardware.

Aside of all the improvement, we implemented a full hydrodynamic radius distribution analysis into SCOUT DPS®. Once, the system is calibrated by a nanoparticle standard, this evaluation does not need any extra work ahead of the separation, but calculates the Rh distribution even for extremely low concentrated samples or for samples smaller than 10nm. In many cases, this feature is capable to compensate a QELS detector, with a sales price ~40k€.

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3 Introduction

The aim of this deliverable is to create a simple cost-effective FFF System which covers all demands of recent nano-science. We have asked the consortium partners in a survey, what the optimization of cost-effectiveness particularly comprises from their point of view. This technical report refers to all the modifications, improvements and inventions making the FFF more effective in its daily use.

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4 Cost effective FFF System

4.1 Consolidation of the understanding of cost effectiveness

At the beginning of the project, we have started a survey among the Nanodefine partners. The purpose was to create an impression what cost-effectiveness particularly means to the active user base. Participants were requested to carry out a valuation of predefined topics. Maximum 5 points per topic could be awarded for the highest importance. Zero points indicate that the topic is unimportant to the participant.

In addition to their ratings, respondents were free to comment on the individual topics. The Figure 1 shows the results per cent, calculated from the average points per topic.

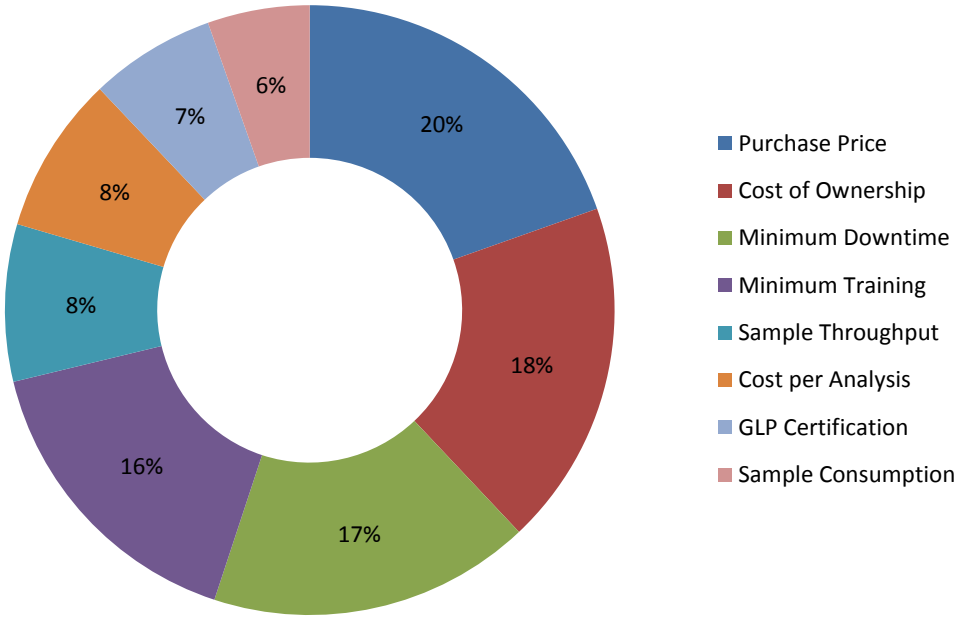

Figure 1: Chart of the main development targets according the survey.

Including the suggestions and advices of the free text, we have been tasked to the following development goals:

1. Reduce the system price of the FFF-System
2. Reduce the running costs of maintenance and service
3. Increase the reliability to decrease downtimes
4. Improve the FFF method development

It has been shown by the free text analysis that the users have considered the point “minimum training" in particular as the improvement of the method development. Therefore, we have formulated it in a more pronounced way as bullet point 4.

4.2 Hardware and Software development

Our first idea was to start coincidently with the milestone 1-3, because all of them deal more or less with cost-reduction. The most expensive components within the chassis of a FFF instrument are flowme-

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ters/controllers as well as motorized needle- and switching valves. We buy all these highly specialized parts ourselves and have almost no influence on the pricing based on the moderate annual output of instruments. On the other hand is a FFF instrument always integrated into system of other expensive HPLC-modules dominating the pricing barrier. Facing both facts turned out quickly, that the tasks could never be solved by simple cost reduction of the parts and assemblies currently under use in the FFF module.

Because we were asked to reduce costs, without decreasing the performance of the instruments, we came up with the idea to introduce a driver platform solution on the software side, supporting HPLC modules in all price segments. While someone needs a sophisticated system with high throughput, others may need low prices only. This approach maintains full flexibility on the technical side and offers the customers buying a HPLC System according their budget situation.

Our new multi-vendor approach comes as new Chromatography Data System (CDS). It implements even today the upcoming RapidControl.Net (RC.Net) industrial standard in a data acquisition system called VOYAGER CDS®. This software can control all hardware modules with RC.Net-compatible drivers present on the market, without dedication to a certain 3rd party manufacturer. Thereby VOYAGER CDS® is the one for all solution without the limitations of other known data systems, but covers the advantage of being intentionally made for the requirements of FFF. We can offer it for a significantly reduced price in comparison the market relevant ones. VOYAGER CDS® is part of our VISION CSH® software package and has been released in January 2017. The number of Rapid-Control compatible HPLC modules has significantly grown over the last months. We expect that all manufacturers will subsequently follow this trend over the next few years.

After an intense market-investigation, we decided to recommend HPLC modules of Knauer GmbH (Berlin, Germany) to all customers with strongly limited budget, because they offer a set of minimalized modules, which excellently fulfil the basic requirements of FFF. We have already created a RC.net compatible pump driver for working package D5.4. A driver for an uv-detector is planned. Using robust standard modules may save up to 50% of the HPLC costs. Using VOYAGER CDS® (Figure 2) will save another 3000 Euro for the HPLC platform solution.

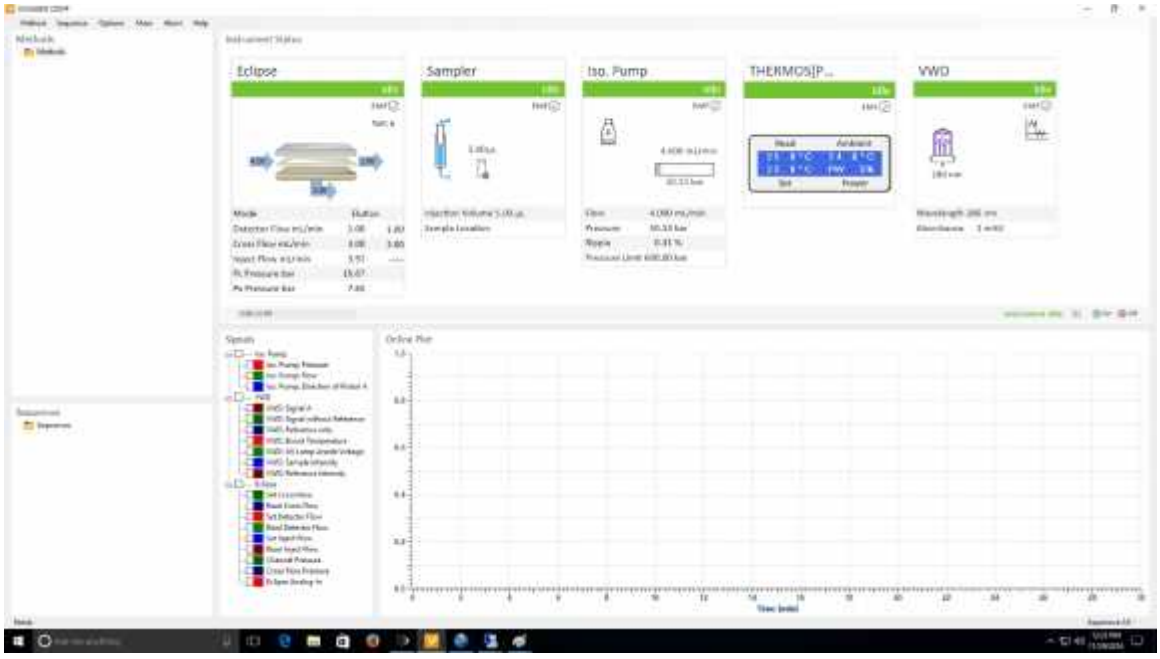

Figure 2: Screenshot of VOYAGER CDS® controlling an HPLC with pump, autosampler, uv-detector, Eclipse FFF and thermostated cabinet.

Another task (bullet point 4), given by the user consortium, is to improve the development of the FFF separation method. This aim is most likely heading to the seamless and effective use of the instrument on its daily base.

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Since the FFF separation is effected by a huge set of variables, e.g. different flow rates, membrane properties, buffer conditions, temperature and many more, beginners are doing hard in predicting the influence of certain method-changes to the expected result. This may leave the impression of getting lost in the mass of potential influencing variables and ends up in try and error experiments. Up to now, method development was strongly dependent on the experiences and skills of the scientists themselves. Thus, we understand cost-effectiveness with regard to the method development as the avoidance of unnecessary time- and material-consuming experiments.

Because we cannot change physics, we cannot reduce the relevant influencing parameters for the separation. Fortunately, the result of a FFF experiment can be precisely calculated based on mathematical expressions in extremely good agreement of theory and real life measurements.

We have already patented an algorithm in 2007, and came up with a prototype software product in 2011. Because of some technical limitations, this early approach did never meet the requirements of the customers with respect to customer-friendliness and convenience. Basically these limitations were caused by third party data acquisition programs, without any chance for improvement from our side. The development of VOYAGER CDS® has significantly changed our possibilities, since we are able to modify the data collection and the inter-program communication according the needs of seamless workflow in method development.

The result of the development is the new SCOUT DPS® (Data Processing Software) software. We have reprogramed the user-interface, the data evaluation procedure as well as the complete data management from scratch. Furthermore, the complete new methodology guides the customer step by step through the process leading to a clearly structured and comprehensive result collection.

SCOUT DPS® can be divided into three main tasks:

- Simulation of chromatograms based on our FFF algorithm without need for prior experiments (Figure 3): This mode is intended for creating virtual experiments, dry testing and “what-if analysis”. An unlimited number of comprehensive simulations for one virtual sample are possible. This is particularly necessary, if more than one separation strategy should be explored for one sample.

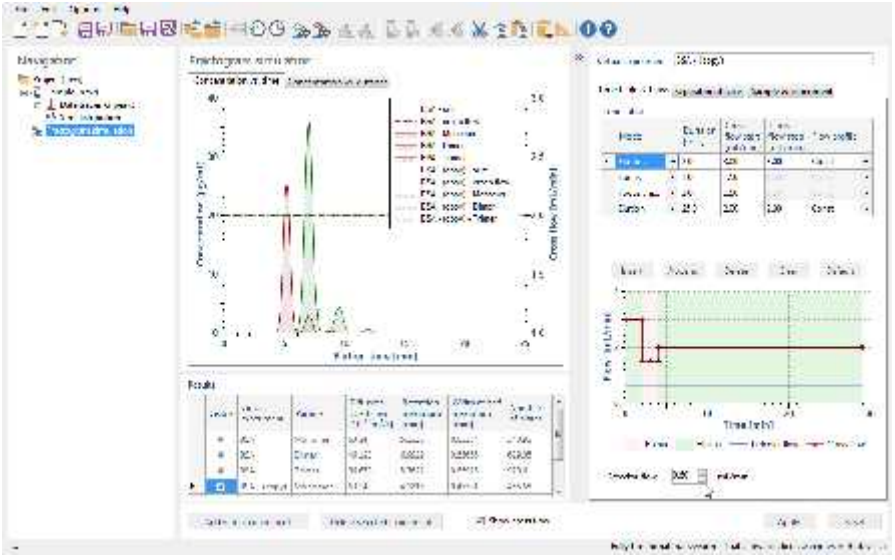

Figure 3: Screenshot of fractogram simulation mode.

- Prediction & optimization of FFF fractograms based on real experimental data: SCOUT DPS® directly reads-in the measurements from VOYAGER CDS® or any other generic data source. Users can optimize the FFF method by simple drag-and-drop operations while SCOUT DPS® directly reflects the inputs by displaying the expected chromatogram (Figure 4). Since all changes are calculated and displayed in real time, one can study the influence of every physical property upon the separation power. Users may iterate the separation parameters as long as it is needed

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to improve the separation. Once, the optimized method is found in the virtual world, SCOUT DPS® converts all information into a real HPLC method, ready to be executed by VOYAGER CDS®. This iteration process between real experiment and virtual improvement/prediction can be repeated until maximum separation strength for the sample is achieved. The software guided method development is of great help especially for beginners without decades of experience in behind. The prediction mode supports branching off an unlimited number of virtual methods for comparison and tracing different separation strategies.

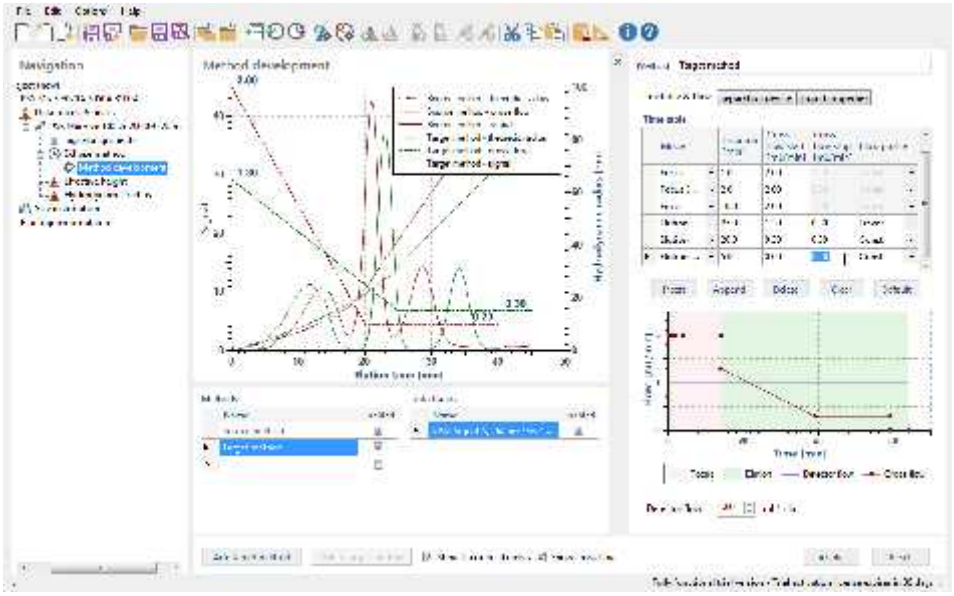

Figure 4: Screenshot of method development mode.

- Data processing & analysis of real FFF experiments: one of the most powerful improvements is the evaluation of the collected data to calculate the hydrodynamic radius distribution for a given separation or even virtual ensemble. This converts the FFF from a separation technique into a detector for hydrodynamic size distributions (Figure 5).

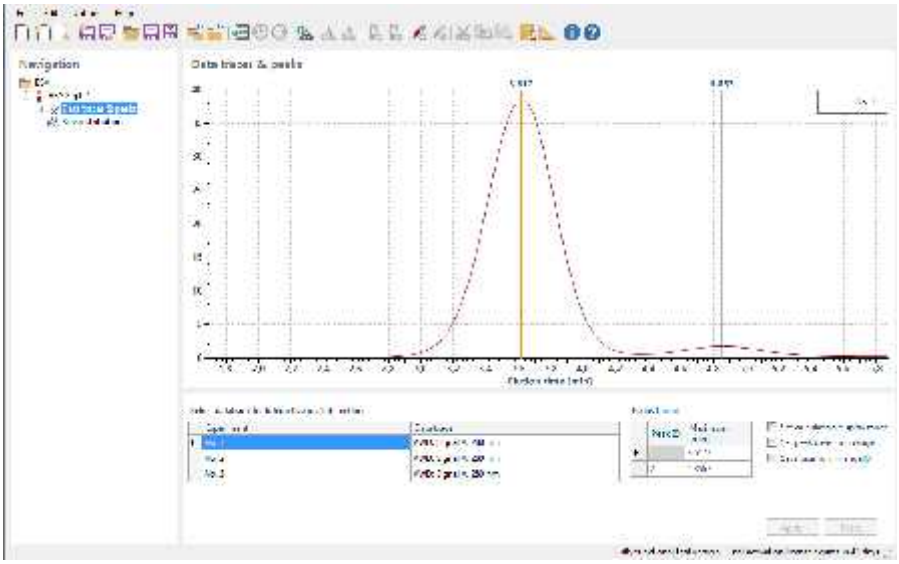

Figure 5: Screenshot of size distribution analysis.

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4.1 Example of simulation, method development and calculation of size distribution for a mixture of nanoparticles

The following chapter shows the complete course of a sample measurement starting from a simulation based on a rough estimation of the radii, which leads us to the first experiment. After discussing the results (light scattering), we turn to the method optimization and discuss the improved results using the predictions and practical MALS measurements. At the end of the evaluation series it is shown how to calculate the hydrodynamic radii of the individual fractions only on the basis of the UV measurement using the SCOUT DPS® software.

4.1.1 Simulation and initial guess

The first step is to simulate a fractogram with the smallest and largest assumed radius of the particle distribution within a suitable time range in combination with sufficient resolution by adjusting the channel and flow properties. In this specific case, we choose 10 nm as smallest and 50 nm as largest radius while applying a cross-flow gradient decreasing from 2 mL/min to 0.2 mL/min within 20 min and afterwards maintaining constant at 0.2 mL/min thru 40 min at a detector flow of 1 mL/min (Figure 6 and Figure 7).

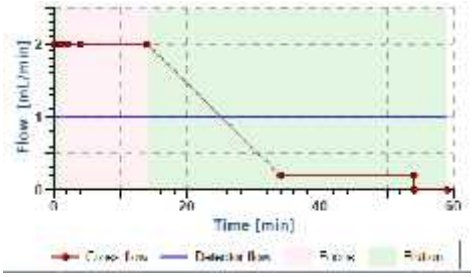

Figure 6: Initial flow diagram.

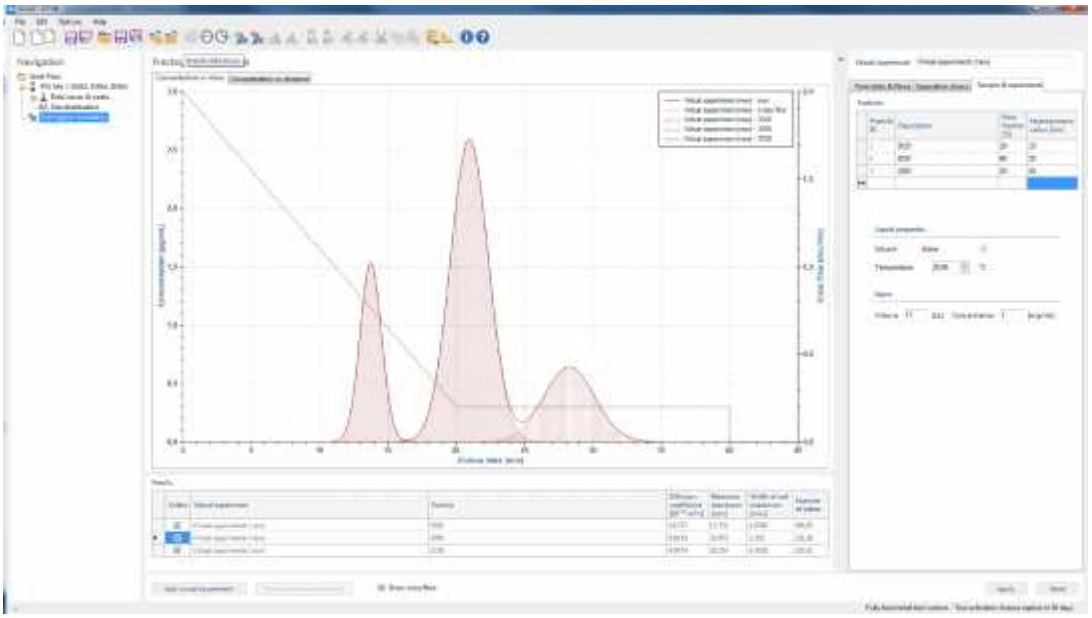

Figure 7: Fractogram simulation based on our initial guess regarding size.

4.2 Running the first experiment

The first real experiment is performed based on the settings of the simulation. We have used a standard uv-DAD detector of Agilent Technologies and a Wyatt Technology DAWN HELEOS II Multi Angle Light Scattering instrument for recording the data. MALS results were evaluated in Wyatt Technology ASTRA 7.0.1 in parallel to the SCOUT DPS® evaluation.

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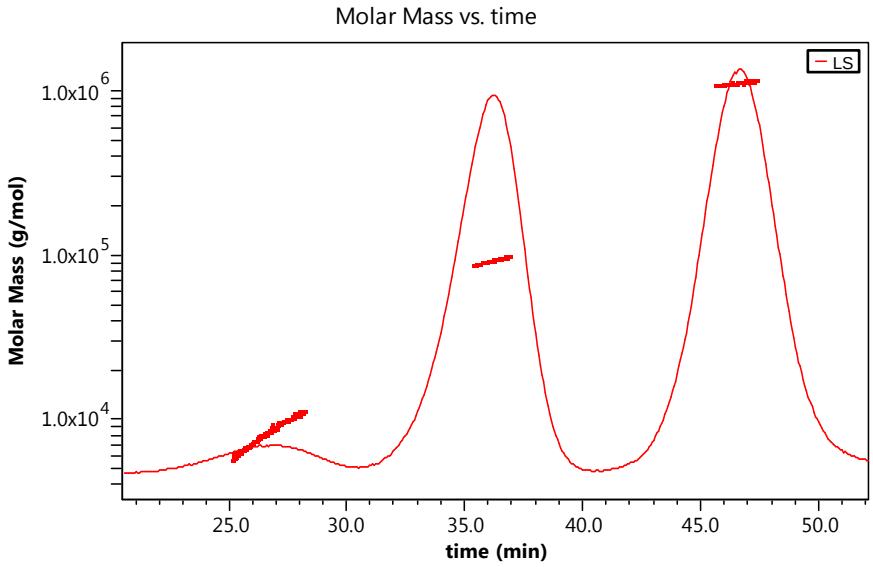

Figure 8: Fractogram as ESI Graph of the ASTRA Software.

Please note that in FFF, the time counting always begins with the beginning of the elution phase. Since we have planned 14 minutes for the pre-elution as well as for the focussing, we have to shift the ASTRA chromatogram at this time. Our sample mixture is a trimodal distribution that contains three nearly monodisperse particles with hydrodynamic mean radii of 11 nm, 23 and 51 nm. The observed retention times of the real experiment and of the simulation agree really perfect (Figure 8 and Figure 9).

After first review of the results in ASTRA, the experiment is imported to the project for comparison with the simulation and further method development.

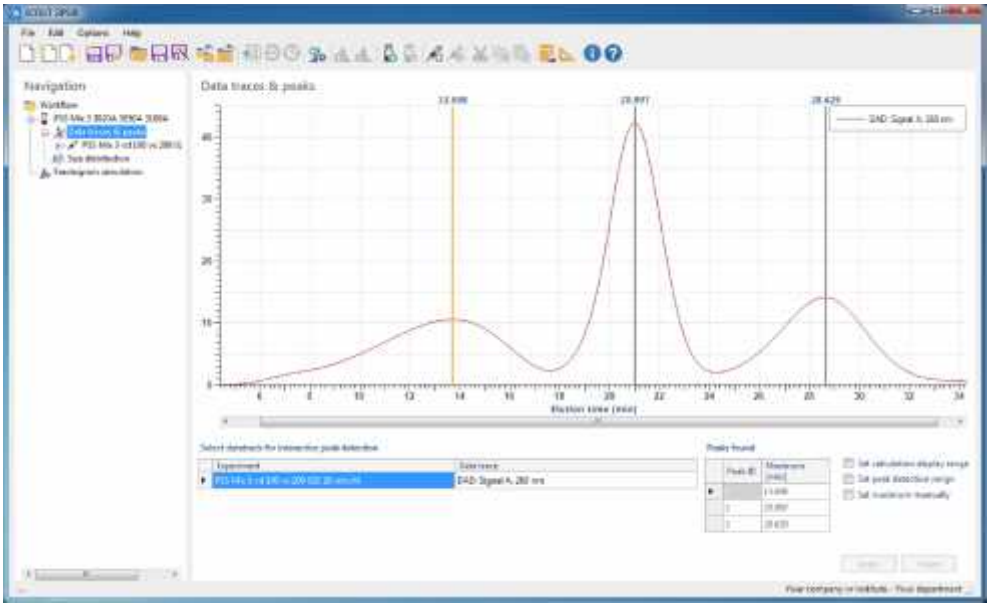

Figure 9: First Experiment loaded into SCOUT DPS®.

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For comparing the results of the simulated and actually performed experiments, it is necessary that both methods use the same effective channel height. Thus, a determination of the actual channel height has been carried out prior the evaluation. Based on this beneficial result we can proceed with the method development.

4.3 Method Development

The purpose of the method development is to improve the separation of the real experiment in terms of resolution between the distinct peaks by adjusting and comparing method parameters of several methods based on the actually calculated size distribution according our needs. The method development module of SCOUT DPS® uses identical controls than our hardware plugins, but has the advantage of calculating and displaying each parameter change in real-time (Figure 10). Basically, the method development mode supports all settings, which can be changed in real as well. This means that the results can also be converted to completely different separation channels (Figure 11).

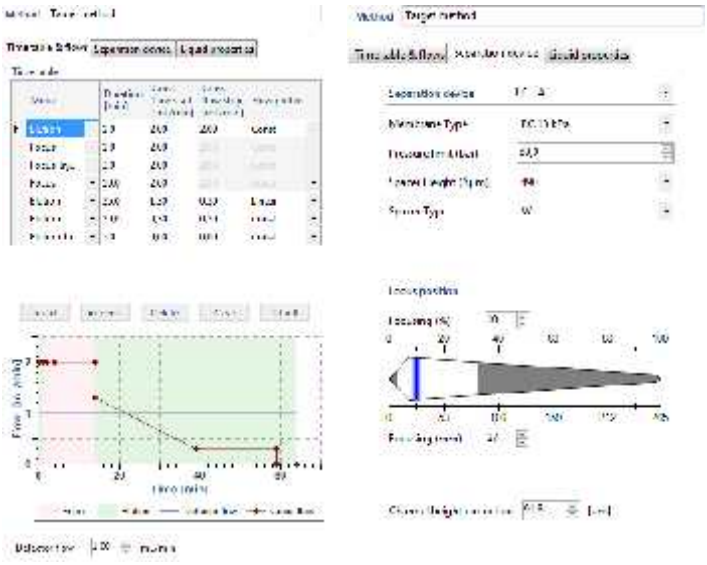

Figure 10: Method development module of SCOUT DPS®.

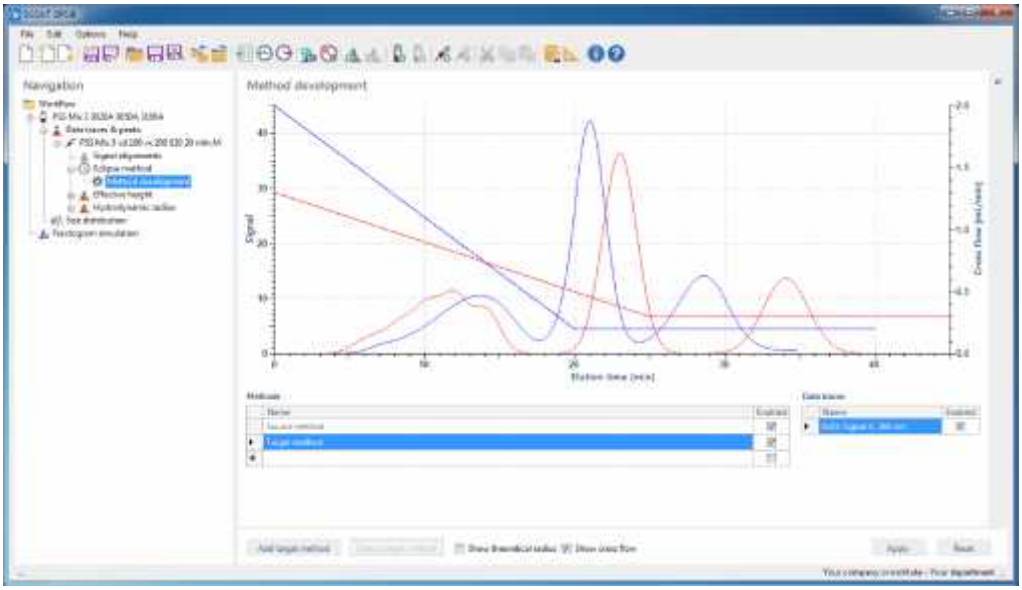

Figure 11: Method development – the blue line is real experiment, while the red plot represents the predicted change by the improved method. Both crossflow curves were superimposed on the respective fractograms as dashed line.

The simulation predicts that increasing the length of the cross-flow gradient in combination with a higher final value will increase the separation performance despite the starting value of cross-flow is decreased.

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Once, the result of the separation can no longer be improved by modification, the optimized method comprising flow program and all channel parameters can be exported to VOYAGER CDS® and run on the real FFF system (Figure 12). Method optimization and real experiments can be iterated until the final result meets the requirements.

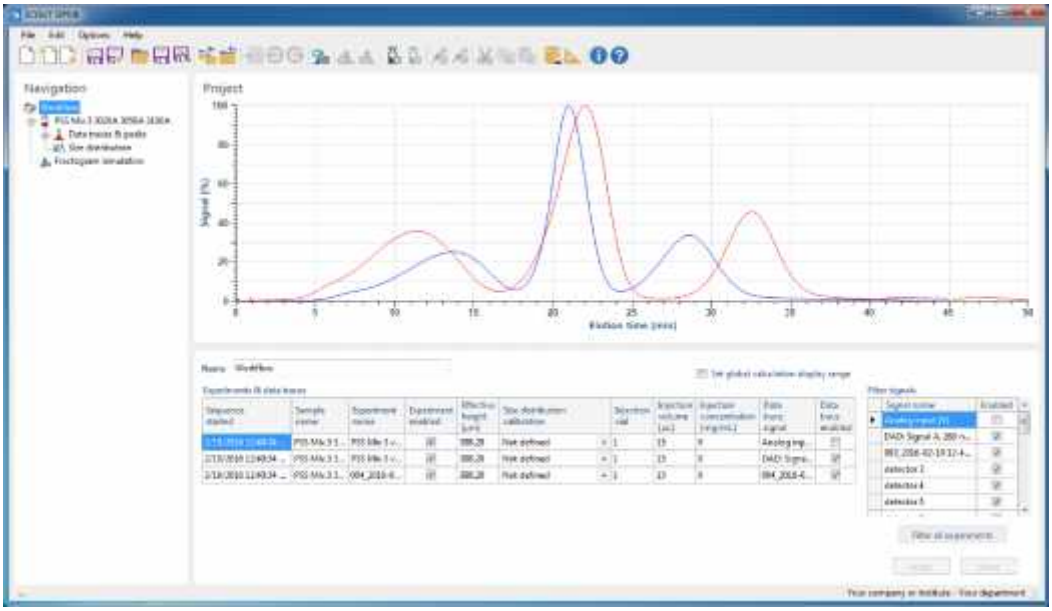

Figure 12: Improved final experiment (red) compared to the initial one (blue).

4.4 Size Distribution

If the results meet the requirements regarding the separation performance, one can proceed with the size distribution of your sample, which can be calculated according to FFF theory and compared with a size calibration, either based on hydrodynamic radius or molar mass standards. In the example below, the size distribution based on FFF theory is in very good agreement with the nominal values. The calculation is done only by one mouse-click without any extra input or assumptions. The peaks defined during the evaluation are labelled by the correspondent R_h in nanometers, but the size distribution is continuously calculated and thereby available for every slice of the experiment. This is of great help when analysing brought distributed sample fraction. Because size distribution is calculated even for virtual samples and simulations, one can decide to optimize the method according the predicted uv-signal (Figure 11) or the radii distribution (Figure 12). Both ways will guide the customers to the most favourable separation method.

Three different Nanosphere™ particles have been mixed for the experiments above. The Table 1 compares the experimental results of Figure 13 with the manufacturer's reference values in terms of sample diameter. Please remind that all these result were achieved without using a Quasi Elastic Light Scattering (QELS) instrument.

Table 1: Comparison of calculated sizes based on FFF measurements and reference values.

Sample	Calculated by SCOUT DPS®		Reference diameter
	radius	diameter	
NANOSPHERE™ 3020	10 nm	20 nm	(21 ± 2) nm
NANOSPHERE™ 3050	25 nm	50 nm	(46 ± 2) nm
NANOSPHERE™ 3100	51 nm	102 nm	(102 ± 3) nm

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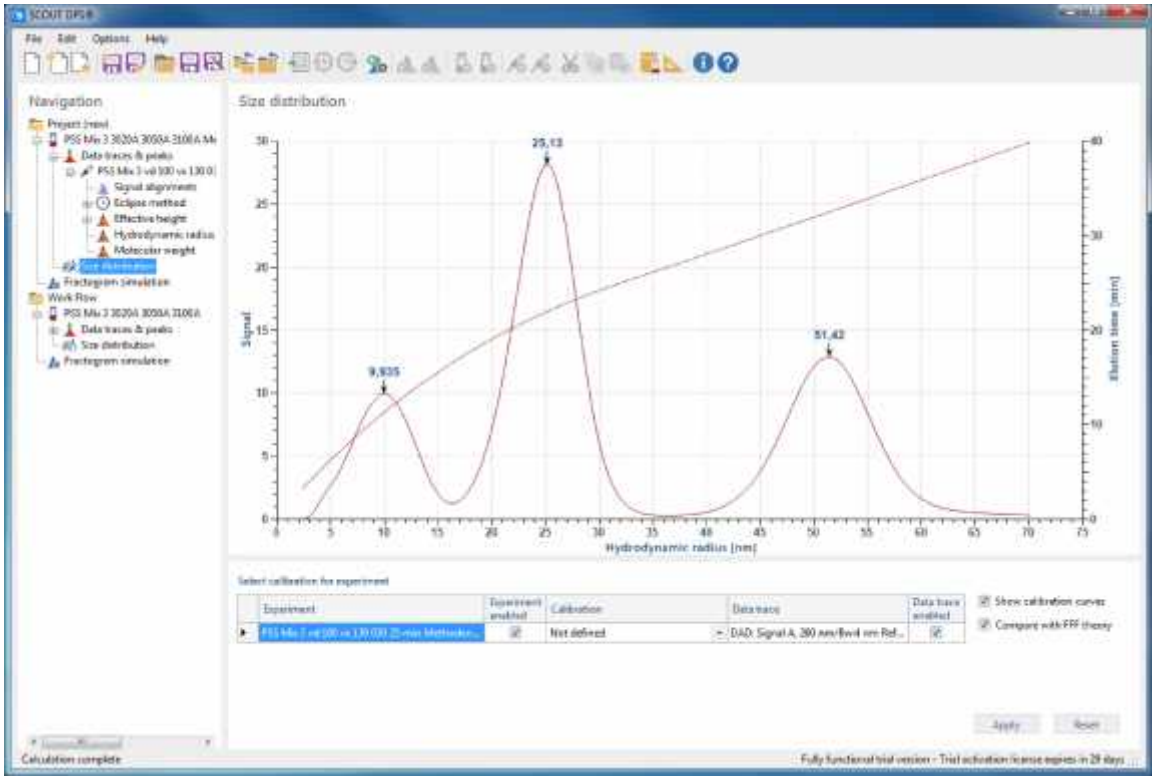

Figure 13: Size distribution of final experiment.

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5 Conclusions

We conclude that there is no other system available on the market, which provides more choice and flexibility with regard to the intended use. There is no other software solution available, which can compensate an application chemist in a better way for method development. No FFF system has ever supported R_h detector functionalities.

We are sure that we have succeeded in finding effective solutions for the four most serious concerns of our consortium partners with the new developments presented by this report.

We conclude with regard to cost-effectiveness, that customers never got more FFF separation power per Euro spend.

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6 References

Marketing brochure VISION CSH®, Superon GmbH ©2016-17

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