High Throughput Screening at the Nano Scale

By Juerg Gentsch and Oliver Bruttger

ABSTRACT

A unique approach to master sample screening at the nano scale was launched as a joint venture project between Evotec Biosystems AG Germany and Novartis Pharma AG. The application, which provides a fully automated nano-screening platform, is composed of three module units: HPLC Nano-Fraction Collection, Micro to Nano Pipetting, and Screening. Process simulation and 3D design tools were used to evaluate not only the most performing module design, but also to reach an outstanding automation and robotic concept. The uniqueness of this application is the combination of miniaturized assay format and single molecule detection read-out, having major beneficial consequences for assay biology. Miniaturization of assays which have been shown to lead to significant savings in assay reagents and test samples. An additional benefit is the physical layout of the 2080-well Nano-Titre-Plate (NTP), which enables screening of rare compounds at low volume and an easy and fast shipment of chemical libraries to different screening sites.

INTRODUCTION

The permanent demand of higher screening throughput by increased competition and financial pressure imposes not only challenges to master new technologies, but also to apply new strategies to enable application development and implementation within a reasonable time frame into an existing screening environment. Screening automation by means of stand-alone workstations, large scale integrated systems and a number of robust standard solutions have a major impact on throughput improvement, but they seldom provide a significant contribution on reagent conservation. However, about 75% of the screening costs are related to the consumption of reagents. Any reduction in the cost of assay chemicals, standards and controls is therefore more than desirable. While a move from 96– to 384– well plates requires in most cases only moderate modifications to existing equipment, new technologies and instrumentation will be necessary to process 1536-well plates. This is particularly true on the liquid handling level, where volumes from micro- to the nano liter scale have to be mastered. An increase in sample plate density provides, apart from less reagent consumption, savings in the field of storage space and labour as fewer handling steps have to be performed. Above all, it opens the area of new read-out technologies on single molecule detection. The question whether one should move or stay on a 96 / 384-well plate technology or go from 96 / 384- to 1536-well plate format, or jump directly into the bio-chip technology depends, apart from the financial investment, mainly on the approach of assay miniaturization, assay compositions, sample detection methodology and the high throughput screening philosophy. This paper outlines the technical path from design to manufacturing of a fully automated platform for miniaturized screening.

DESIGN APPROACH

System design and development of any new technology platform demands not only expertise in different fields, but also a very detailed project scope and plan, technical specifications to be met, decision and exit points of the different design approaches. One should, whenever possible, integrate elements or devices of a proven technology into a new system concept to lower the overall project risk. Very often the focus is only set on the application point, neglecting integration, workflow, information technology and logistical aspects. The set-up of a simple block diagram (**Figure 1**) can help to draw attention to the different interface requirements and operating interactions. Furthermore, one should analyes in detail the requested know-how, expertise and the corresponding responsibility to enable the most suitable technique to be applied. This is very often a time consuming task as the ideal balance between internal and external research and development activities has to be found.

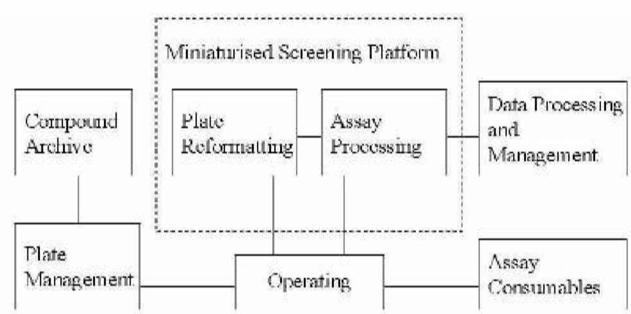

Figure 1 Basic Block Diagram of a Miniaturized Screening Environment

Our basic project target was to design and build-up a platform for miniaturized screening, which could handle an initial throughput of at least 20,000 samples within 24 hours. The co-operation with Evotec Biosystems proved to be an ideal combination as we could bring in all our expertise and know-how in lab automation, robotics and information technology, while they had already gained know-how in nano pipetting, dispensing, assay down scaling, NTP development and expertise in sample reading based on Fluorescence Correlation Spectroscopy (FCS). To manage the different development disciplines in parallel was one of the key challenges in this project.

From the automation point of view, a first layout concept for miniaturized screening had to be established, which was composed of a robotic and conveyer system for plate handling, two pipetting units for micro to nano reformatting, four nano dispenser assemblies and a set of devices for storage, incubation, evaporation and a FCS reader instrument for plate reading (**Figure 2**.) The main objective of this first design concept was to keep the layout of the platform at a very small footprint. Not included herein is the HPLC nano-fraction-collection process, as this task is performed in a different building.

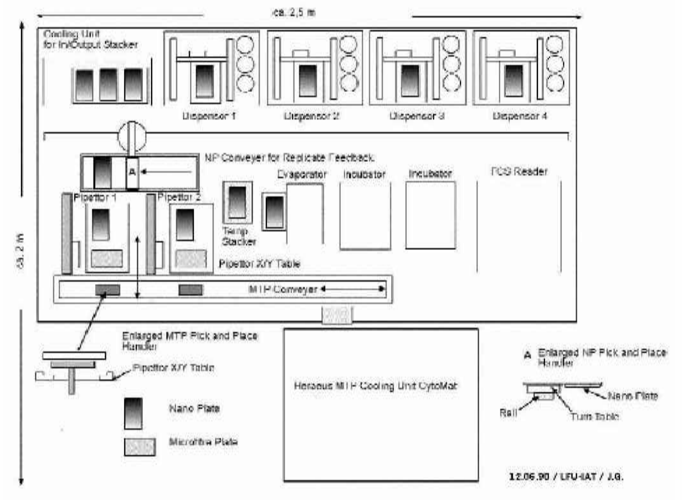

Figure 2 First Layout Concept for Miniaturized Screening

Proof of concept and the evaluation of alternatives was the key element of the subsequent project activities including the aspects of system set-up time, performance, system autonomy and the corresponding logistic consequences for plate management and assay consumables. *Extend*, a simulation software tool of Imagine That Inc. (San Jose, CA 95119-1353 USA), was used to analyse the different requirement profiles.

CONCEPT OPTIMIZATION BY SIMULATION

The use of simulation tools for process optimization, re-engineering tasks or performance analysis of new manufacturing plant floors is very common in manufacturing industry—a process which has also slowly emerged in the field of lab automation over the past few years, mainly for sample process scheduling. Workflow–and–sample process optimization (**Figure 3**) is a valuable task in any screening performance analysis. In particular, no additional investment in equipment, apart from the simulation software tool, has to be initiated.

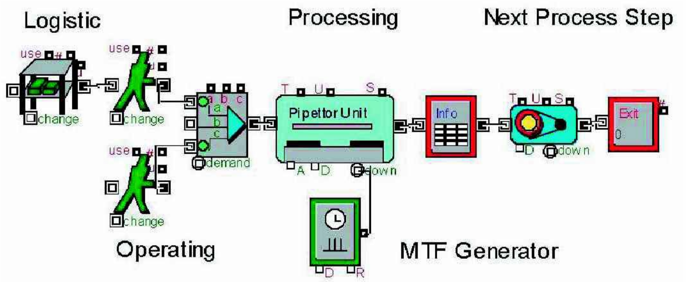

Figure 3 Basic Workflow Simulation

The *Extend* simulation set-up is composed of a set of elements, which are retrievable from a library (**Figure 4**) and assembled, according to the desired process or workflow. A retrieved element can, in case of a specific application requirement profile, simply be modified by an editor or established from scratch on a C programming level. Each element is equipped with a set of pull down menus to perform parameter set-up or performance analysis. To visualise the different work– or process– flows a set of animation symbols can be linked and activated.

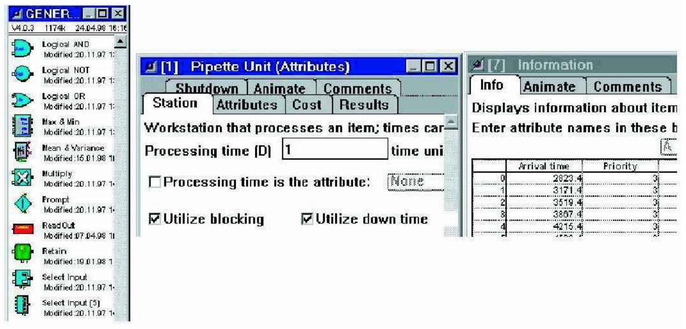

Figure 4 Extend Simulation Library and Pull-down Menus

Keep it simple but effective is an old automation proverb, which one should use as guideline for a first simulation approach of any application. It helps a lot to validate the model before moving into the complexity of parallel tasks or processes to be analyzed. One of the key elements in miniaturized screening is in terms of performance of the reformatting process, as a number of Micro-Titre-Plates (MTP) have to be copied on a single NTP. To analyze the different reformatting performance characteristics on a 24 hour basis, we included pipetting constrains, tip cleaning sequences, plate handling interlocks, operating interactions and mean time between failure interrupts in the simulation model (**Figure 5**). Some of the simulation elements are hidden on a second module level to keep screen display and animation simple. A number of simulation runs with different handling concepts had to be performed to evaluate the most efficient automation structure.

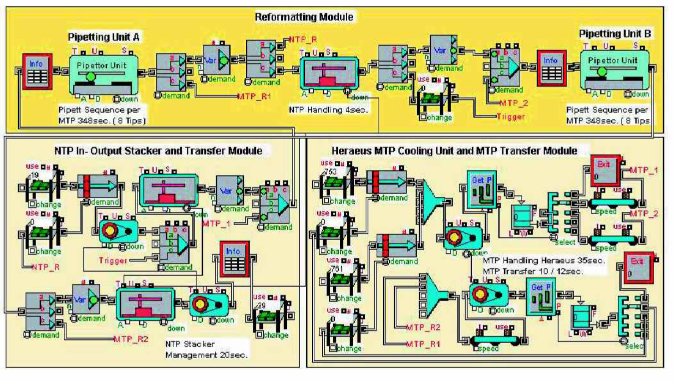

Figure 5 Micro to Nano Reformatting Model

The set-up and performance of the HPLC and Screening Module was analyzed with different simulation models according to their specific profile requirement. However, they have been created with the same set of simulation elements, which not only cuts-down on simulation model set-up time, but also visualizes very clearly where synergies in terms automation components can be used mainly on the level of plate handling, pipetting/dispensing and storage management.

The different simulation scenarios proved that an "all in one" concept was not the favourite solution, although from an economical and space consumption point of view, it was a very attractive one. The decision to move towards two independent module units for micro to nano reformatting (**Figure 6**) and assay screening was based on the view of module integration into the existing screening environment, future extension and flexibility of module set-up and operation.

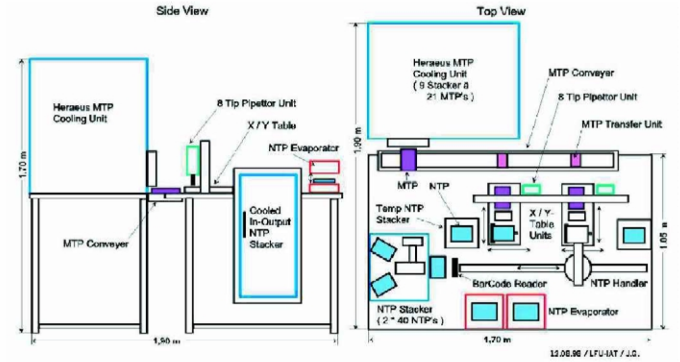

Figure 6 Micro to Nano Reformatting Module Layout Concept

The detail design phase of technical manufacturing plans do very often exclude the end user, as an engineering background is required to understand the interactions of the different elements. User specific modifications or up-grades are, if feasible from the time frame and cost point of view, not possible as long as drawing progress or machine assembling has not reached a status which can be overviewed by the end user. To eliminate the possibility of misunderstandings and to provide a solid ground for module design optimization, we converted the simulated concept drawings into 3D models (**Figure 7**). As a result, a view of "what you see is what you get" can be reached before any manufacturing takes place. An additional benefit of a 3D model is that it can be displayed and checked from different view points on the screen. Furthermore, it enables the designer to optimise module socket space for supporting equipment (**Figure 8**). Although establishing 3D models is a very time consuming task, it reduces the real detail design phase to a great extent as the conversion of 3D elements into manufacturing plans is performed automatically by the design software from *Solid Works*.

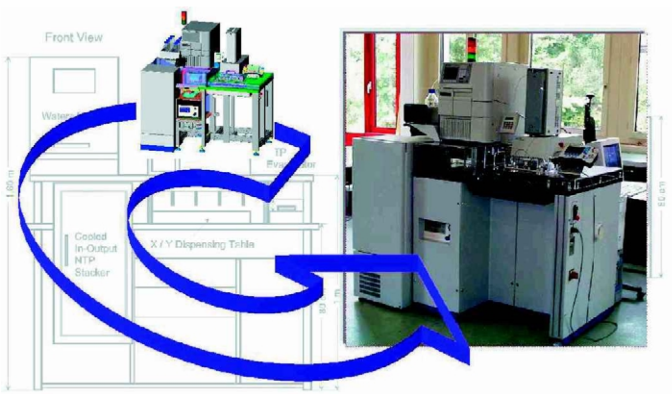

Figure 7 HPLC Nano Fraction Collection Module from 3D Model Design to Manufacturing

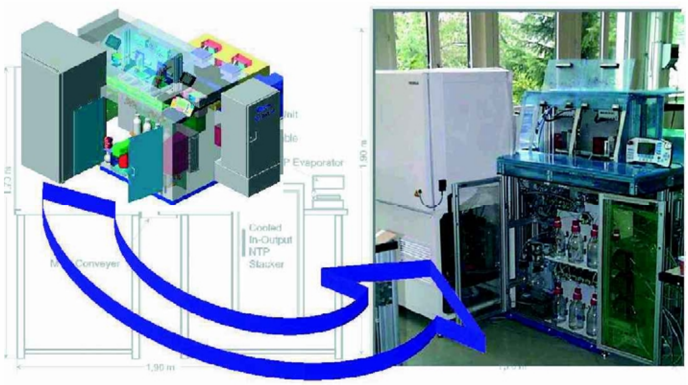

Figure 8 Reformatting Module Micro to Nano from 3D Model Design to Manufacturing

COMMON MODULE ELEMENTS

One of our design strategies was to use common devices and automation elements within the different modules to reduce development time, cost and risk of failure, particularly on the level of liquid handling, pipetting/dispensing (tip and table) and plate transfer. Small in size and fast in performance was the objective for the NTP handler design (**Figure 9**). One of the handler features is, apart from high speed performance, that the plate is covered airtight by the gripper during the handling process to minimize sample evaporation. The same philosophy was applied to the NTP storage devices, which are furnished with an integrated handling system (**Figure 10**) and two removable stacker units for an easy manual loading and unloading task. Each stacker has a NTP capacity of 42 plates.

A critical element in miniaturized screening is, apart from sample evaporation problems, liquid handling at small volumes, above all, pipetting of compound samples and dispensing of assay components at a volume range of 50 to 80nl. Currently there are a number of technologies, such as inkjet, syringe-solenoid dispensing and pin-transfer, on the market that can be applied for nanodispensing. However, in all cases know-how and application experience is necessary to establish a system, which is capable of carrying out pipetting/dispensing unattended without being prone to constant air-bubbles or particle clogging problems or having drawbacks of CV value reproducibility. We applied inkjet technology since Evotec had already gained know-how and experience in this field.

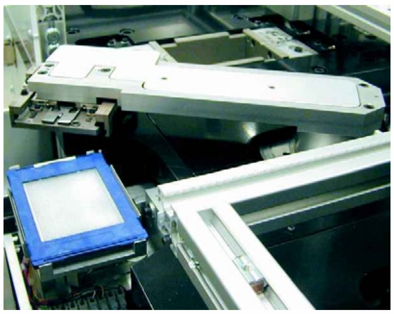

Figure 9 NTP Handler

Figure 10 NTP Storage Unit

a). HPLC Nano-Fraction Collection Module *NACONA*

The module (**Figure 11**) is based on a solid Bosch-profile frame with an integrated NTP handling system, which provides the transfer link to the different NTP sub-module units. An on–board console with a back-illuminated LCD display and a set of programmable soft-keys enables manual control of module commands, the set-up of the different peripheral devices, or display process status and, if necessary, error messages. The heart of the module unit is the composition of the Waters® Millennium 2690 separation module, the column oven and the Waters® 996 photodiode array detector, which are arranged close to the dispenser head to render a fluent and short transfer of eluents in capillary tube connections. NTPs used in the nano fraction process are retrieved from a cooled NTP storage unit, conveyed to a barcode reader for the identification of the plates and then placed on the Nano-Fraction (NF)-table, which is mounted on a X-/Y-table system with a resolution of 50µm. The table can be loaded with up to four NTPs (replicates) and is equipped with sensor devices to control the plate load- and unload status. Nano-Fraction dispensing is performed by a dispenser head assembly unit, which is composed of four dispensing tips and a laser device to enhance the CCD camera supervision of the dispensing task. The unit is located just above the NF-table and enclosed in transparent plexiglas. A– NTP evaporator unit (Barkey® Evaporator), next to the NF-table, manages and controls the evaporation of the NTPs within 15 minutes.

Figure 11 HPLC Nano Fraction Collection Module NACONA

The module is able to dispense HPLC injections from vials into NTPs using up to 1536 wells, which are normally subdivided into 5 sectors of 288 wells each. Space for additional wells for high/low controls or assay references is available as the plate has a capacity of 2080 wells. Each well can be loaded with up to 1.5 microliter. The throughput performance is mainly defined by the separation and collecting process, which is in the area of 10 to 20 minutes per extract. Unattended operating sequences of 24 hours or more are feasible, as the NTP storage device can hold a total of 84 plates. A nano-fraction process sequence is composed of a NTP retrieval from the NTP-storage unit, identification by the barcode reader, loading on the NF assembly, task synchronisation of the Waters® 2690 separation module, injection and separation of the extracts into the wells, as well as the evaporation of the NTPs at the end of the process. The process is controlled by a PC, which is linked to a Screening Information System (SIS) and the Waters Millennium database. Process parameter and nano fraction collection layout are established on the SIS level by means of a graphical user interface, where also sample- and plate identification as well as the collecting time of each fraction are monitored.

b). Micro to Nano Reformatting Module *MITONA*

The module (**Figure 12**) is based on two independent pipetting units for MTP/NTP reformatting. Each unit is equipped with an integrated tip wash station and a pipetting head of 8 tips (**Figure 13**). The unit can handle 96- as well as 384-well plates and transfer more than 100,000 samples per 24 hours in a single or replicate NTP mode with a compound transfer volume of 10- to 600 nl. In case of the replicate mode up to 20 NTPs can be processed in a sequential task. Plate management is performed by an integrated NTP handling system, which provides the NTP transfer link to the pipetting tables, the temporary stacker units for replicate processing, the NTP storage- and evaporator device. A MTP conveyer assembly (**Figure 14**) is responsible for the bi-directional plate transfer to the Heraeus-Kendro cooling unit and the pipetting tables. The correct plate placement on the pipetting table is supervised by a laser beam to prevent a tip crash during the reformatting process. Plate identification is controlled by two integrated bar-code readers for code 128. The Heraeus-Kendro MTP cooling unit provides, in case of 384-well plates, a sample storage capacity of more than 72,000 samples.

However, a successful continuous reformatting process on a 24 hours shift demands not only process supervision by sensor devices, detectors and smart pieces of software on the controlling level, but also a liquid management system for internal and external tip cleaning since up to 60 litres are consumed within one shift. To eliminate supply constraints, the module is equipped with an on– board Millipore Water supply system and a set of bottles to enable a continuous refill and the supply of additives, such as DMSO, without interrupting a running process. Pipetting layout, process parameter and the required process sequence are created and controlled by a PC, which is linked to the SIS. The module is also furnished with two CCD cameras and monitors to supervise the reformatting process.

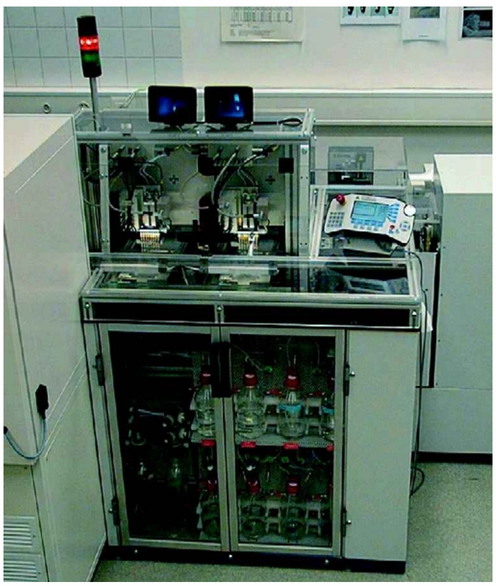

Figure 12 Micro to Nano Reformatting Module MITONA

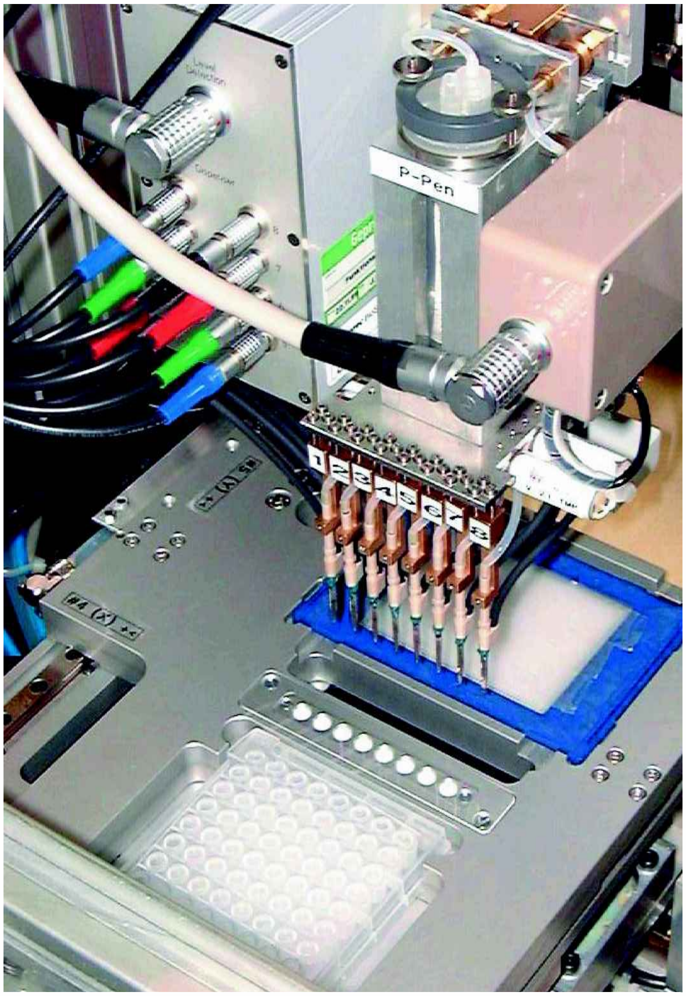

Figure 13 Pipetting Head

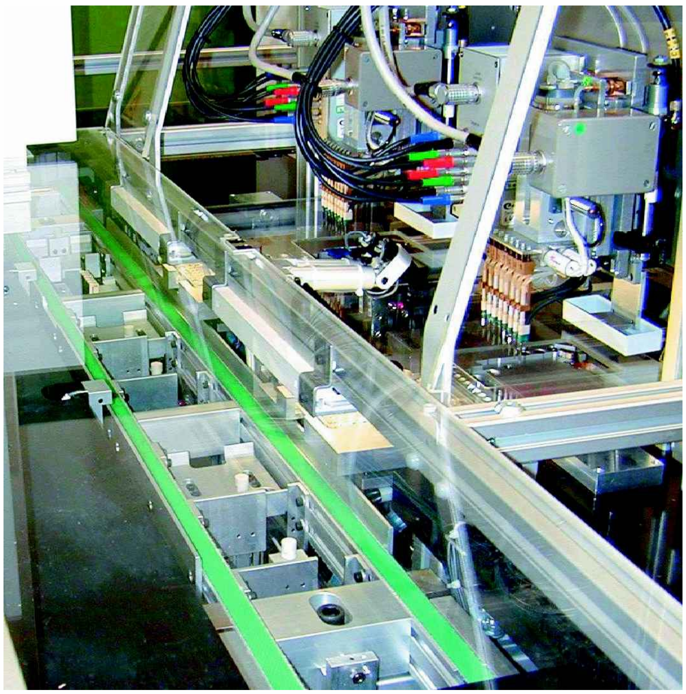

Figure 14 MTP Conveyer

c). Assay Screening Module ***SCARINA***

The screening module (**Figure 15**.) is the heart of the miniaturized screening application. Compound samples or extract fractions, which have been previously applied to NTPs on a Mitona or Nacona level, are transferred in stacker units in a dry or liquid format, depending on the assay or research task. The module is equipped with an NTP storage cooling unit for NTP in- and output with an integrated bar-code reader for code 128, –NTP unit for incubation at 37°C and one for temporary storage with a high humidity climate at 10°C. For assay component dispensing a set of four independent dispenser units can be addressed. Each dispenser unit (**Figure 16**) is equipped with two independent nano pens, which are composed of two dispensing tips, an assay component and tip cleaning reservoir and a CCD camera and monitor system for dispensing supervision. The assay component reservoir is temperature controlled and can handle 4°C to room temperature. The dispensing path and the dispensing parameter are established on a host PC, which is linked to the SIS, and downloaded before the process is launched. Plate management is, as in the case of Nacona and Mitona, controlled by an integrated NTP handling system, which provides the link between the different NTP devices, the dispensing tables and the FCS plate reader. The FCS-reader module (**Figure 17**) enables one to determine the "activity" of test samples towards a given target by various confocal fluorescence read-out techniques .[1,2]

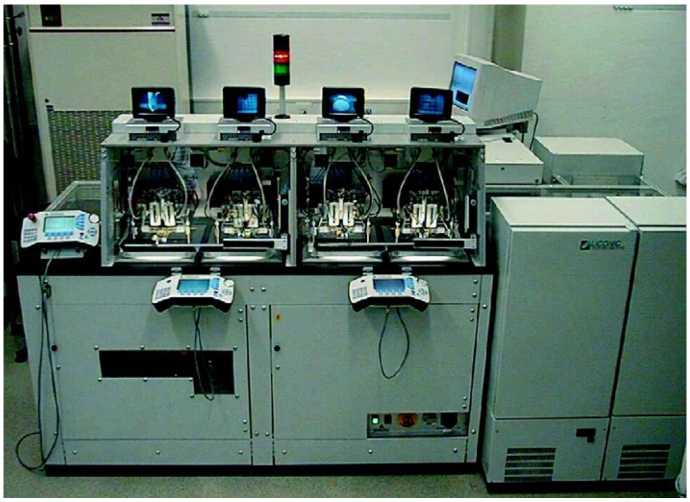

Figure 15 Assay Screening Module SCARINA

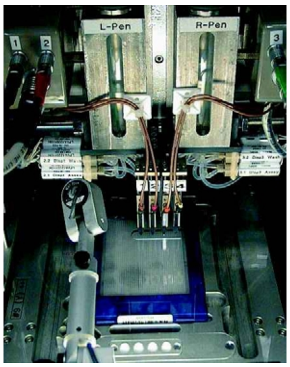

Figure 16 Dispensing Pen A / B

Figure 17 FCS Reader Unit

CONCLUSION

The tremendous progress in evolving technologies demands not only a new implementation strategy to reduce the risk of failures, but above all internal and external co-operations in the field of the different expertises to master these technologies within a reasonable time and cost frame. The presented project demonstrates also very clearly that an interdisciplinary project team is a vital key element for success in such a high tech project.

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AUTHOR BIOGRAPHY

Jürg Gentsch worked as an electronics development engineer for Eaton International, Inland Motors and WER Industrial Drives in the USA, Europe and Middle East before he joined former Sandoz AG (Novartis merger) in 1980. He introduced the first robotic system at Sandoz in 1984 and worked as an automation expert in a number of robotic and high tech Pharma projects. Currently he is responsible for high throughput screening automation at Novartis Pharma Lead Finding Unit.

Oliver Bruttger joined former Sandoz in 1988 and worked as a scientific technical assistant in Pharma research. Today he is responsible for the operating aspects of miniaturized high throughput screening at Novartis Pharma Lead Finding Unit. ■

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CONTACT ADDRESSES

Juerg Gentsch
Novartis Pharma AG / Automation, Information Technology / Lead Finding Unit
juerg.gentsch@pharma.novartis.com

Oliver Bruttger
Novartis Pharma AG / Lead Finding Unit
oliver.bruttger@pharma.novartis.com

Martin Daffertshofer
Evotec Biosystems AG
martin.daffertshofer@evotec.de

Bernard Fiedli
Sysmelec SA
b.friedli@sysmelec.ch

Cosmas Malin
Liconic AG
cma@pingnet.li

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