Biochip Printing using Virtual Instruments

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Fig. 1:   TopSpot System

For its business activities involving biochip technology – including the development, production, marketing and application of biochips – GeneScan Europe AG has coined the term "BioChipnology®". Biochip-based analysis represents the most advanced method to analyse biomolecules, especially nucleic acids. The surface of a biochip generally accommodates a few hundred to a few thousand spots (radius: 100 – 300 µm) with coupled biomolecules . These are created in the form of a matrix ("microarray") using a printing system. This special matrix of biomolecules forms the DNA array, the biochip's sensitive core. The advantages of biochip analysis lie in the synchronisation, miniaturisation and high speed of the entire analytical process. This also entails a reduction in costs due to savings made on materials and reagents. Moreover, biochip analysis boosts the efficiency and precision of the analytical process. GeneScan Europe AG was among the first companies to move into the field of microarrays ("microarray" = defined micro-arrangement of biomolecules in matrix form), which are often also referred to as DNA arrays or biochips. In the microarray sector, GeneScan specialises in low and medium spot density biochips. Low-density biochips include all those that accommodate between ten and several hundred different biomolecules. The medium-density range can accommodate up to 10,000 different biomolecules functioning as information carriers.

Biochip analysis is a highly innovative method of modern molecular biology. The core of this analytical technology are "biochips". These consist of a carrier, made of glass or other materials, on which biomolecules are fixed in high numbers and densities in a defined microarray. Depending on the purpose of the test, the surface can contain several hundred or several thousand spots with linked biomolecules. Each spot represents the equivalent of a conventional analysis in a test tube. The matrix of spots applied to the biochip reproduces each of these analyses several times – up to a total of three or four – and thus increases the reliability of the test result.

The advantages of biochip analysis compared to conventional analytical methods lie in the synchronisation, miniaturisation and acceleration of the analytical process, and are also reflected in the material savings caused by the minimisation of sample volumes and reagent use, along with an associated reduction in costs. Biochip analysis considerably boosts the efficiency and measuring accuracy of the analytical process, and also brings about greater flexibility and mobility. Depending on the density of the media applied to the carrier materials, several thousand analyses can be accommodated on a single biochip.

Areas of application for biochip technology include the analysis of plant and animal genetic material, the analysis of pathogens and infections, human genetic questions, gene activity studies and the investigation of differences in DNA sequences. GeneScan develops, produces and markets biochips equipped with different features, thus covering a wide range of areas – including food and environmental analysis, medical diagnostics and scientific research.

In biochip analysis, the “key and lock principle” of complementary hybridisation is utilised. Freely-moving molecules from the test sample that are marked with a fluorescent dye (“key”) are brought into contact with specifically designed and synthetically produced biological probe molecules (e.g. genetic material, DNA), which are fixed to the carrier (“lock”). When the identical probe molecules of a spot match up with and capture their complementary binding partners for the test feature from the analysis sample, the linked fluorescent dye is likewise immobilised on the chip. The respective “positive” spot on the microarray lights up in the subsequent detection reaction. Via hybridisation a specific molecule in the test sample (“key”) can be identified if it binds to the complementary molecule (“lock”) which is immobilised on the chip in the form of a synthetic probe with a defined structure.

The TopSpot® printer is a patented, contact-free printing system for biomolecules. The printheads manufactured by silicon micromachining technology permit simultaneous printing of 96 different media. Top-quality microarrays can be produced because each printing module reproduces the whole array without mechanical displacement and applying precise dosage volumes.
(fig. 5,6,9)

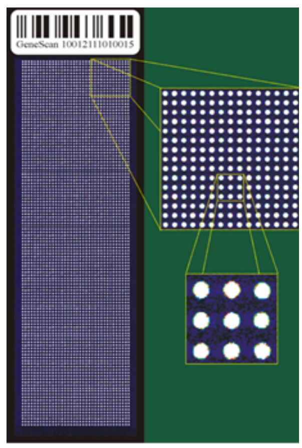

Fig. 2-4: Printing modules and print patterns

With the TopSpot® system (TopSpot® D) GeneScan, together with its partner SYSTEC GmbH (www.systec-gmbh.com, Germany), have developed a model that has been specifically designed to meet the requirements for high-throughput production of biochips.

Hardware

TopSpot® D is a high speed and low cost microarrayer (fig. 1, fig. 2) that leverages latest PC and DAQ technologies. All DAQ, IMAQ, signal conditioning, and motion components are located in a Coolermaster aluminium PC case. The system features three power supplies (Enermax ATX power supply 430W, 24V DC/DC converter, high voltage power supply). The Enermax device supplies the mainboard, plug in boards, drives, and the motion controller with voltage.
The motherboard is a Pentium IV based ASUS P4C800 i875 Canterwood mainboard. The Intel 875P chipset is Intel's latest desktop chipset products to integrate the most advanced PC technologies available today, and to boost the system performance to the next level through the state-of-the-art 800MHz front side bus.

Specs
CPU Intel Pentium 4 / Celeron / Prescott up to 3.6+ GHz
Intel Hyper-Threading Technology ready
Chipset Intel 875P MCH, Intel ICH5R
FSB 800/533/400 MHz
Memory Dual Channel DDR400, 4 DIMM Sockets, Max. 4GB
Expansion Slots 1 x AGP 8X, 5 x PCI
1 x ASUS WIFI connector for Wireless LAN upgrade
Storage ICH5R:
2 x ATA100, 2 x Serial ATA with RAID 0
Audio ADI AD1985 6-channel CODEC, Audio ESP
LAN 3COM 3C940 10/100/1000 Base-T, VCT
AI Features
AI Audio, AI NET, AI Overclocking
AI BIOS: CrashFree BIOS 2, Q-Fan, POST Reporter

The graphics subsystem is based on an ATI Radeon 9600 Pro chip with DVI interface that is directly connected to a digital 17” TFT display with integrated speakers.

The piezo actuator is controlled via NI PCI 6711 High Speed Analog Output Card and an APEX high voltage amplifier. The PCI 6711 and the APEX HV AMP (integrated in a 5,25” device bay) are connected together with the SYSTEC I/O subsystem that´s located in a 5,25” bay. Each liquid can be printed using different waveforms. Upon activation of the actuator, droplets of the printing solution are ejected out of the nozzles. The droplet volume is about 1nl and can be adjusted using different waveforms.

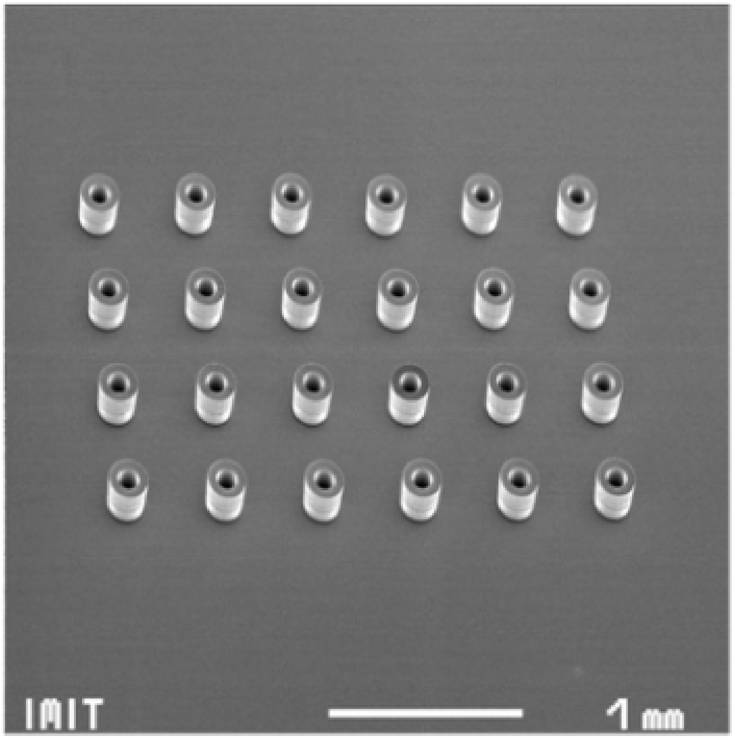

Fig. 5: Nozzles

All waveforms can be manipulated with an LabVIEW teaching subsystem. The waveforms and all relevant test data are stored in a database. The customer can decide which database to chose.

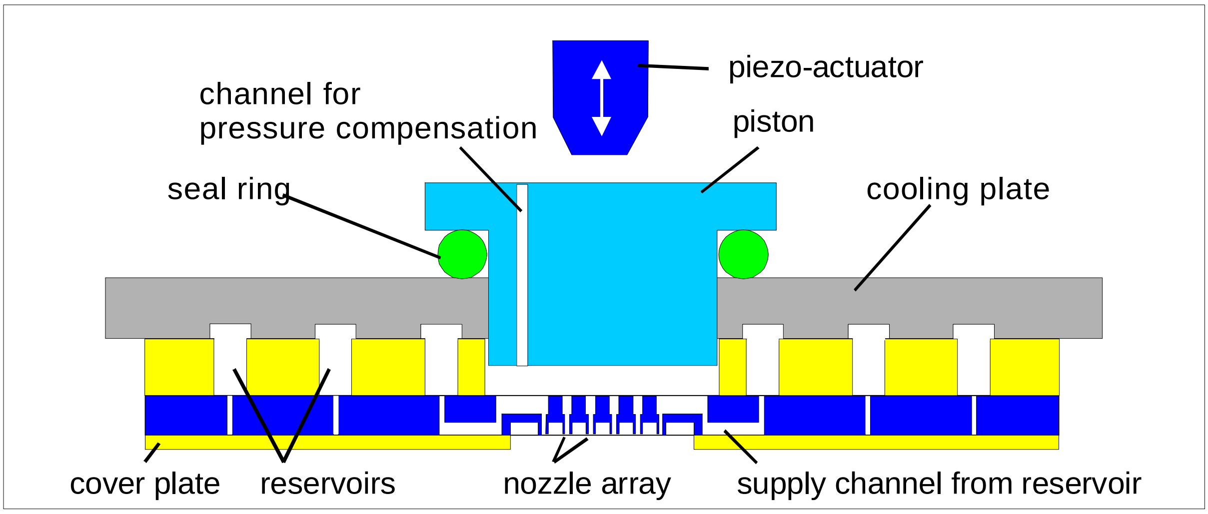

Fig. 6: Print head schematics

The printing head together with a SONY firewire color camera DFW-SX900 and the programmable illumination subsystem (SYSTEC I/O) is mounted on the z-axis of the microarrayer.
A new-age digital camera that produces never-before-seen levels of detailed, uncompressed images, Sony's DFW-SX900 high-resolution color C-mount digital camera is in a league of its own. The camera incorporates a 1/2" Interline (IT) progressive scan 1.45 million-pixel CCD and includes an IEEE-1394 digital interface. A compact and lightweight design makes the DFW-SX900 easy to install and ideal for a variety of quality critical applications, such as machine vision, photo ID badging, microscopy, and high-resolution display.

The dropsizes are 100% verified via image acquisition using the NI IMAQ library. The camera is controlled by LabVIEW VI´s using the NI IEEE1394 driver. The NI-IMAQ based LabVIEW algorithms are responsible for biochip analysis.

The air conditioning subsystem is essential to prevent the evaporation of the droplets. This subsystem is fully controlled using a low budget NI 6014 DAQ device and the SYSTEC I/O subsystem.

The glass plates are positioned using a cross table with a movement range of (X,Y) 300 by 300 mm. The cross table and the z axis are fully controlled using a Lang LSTEP system.

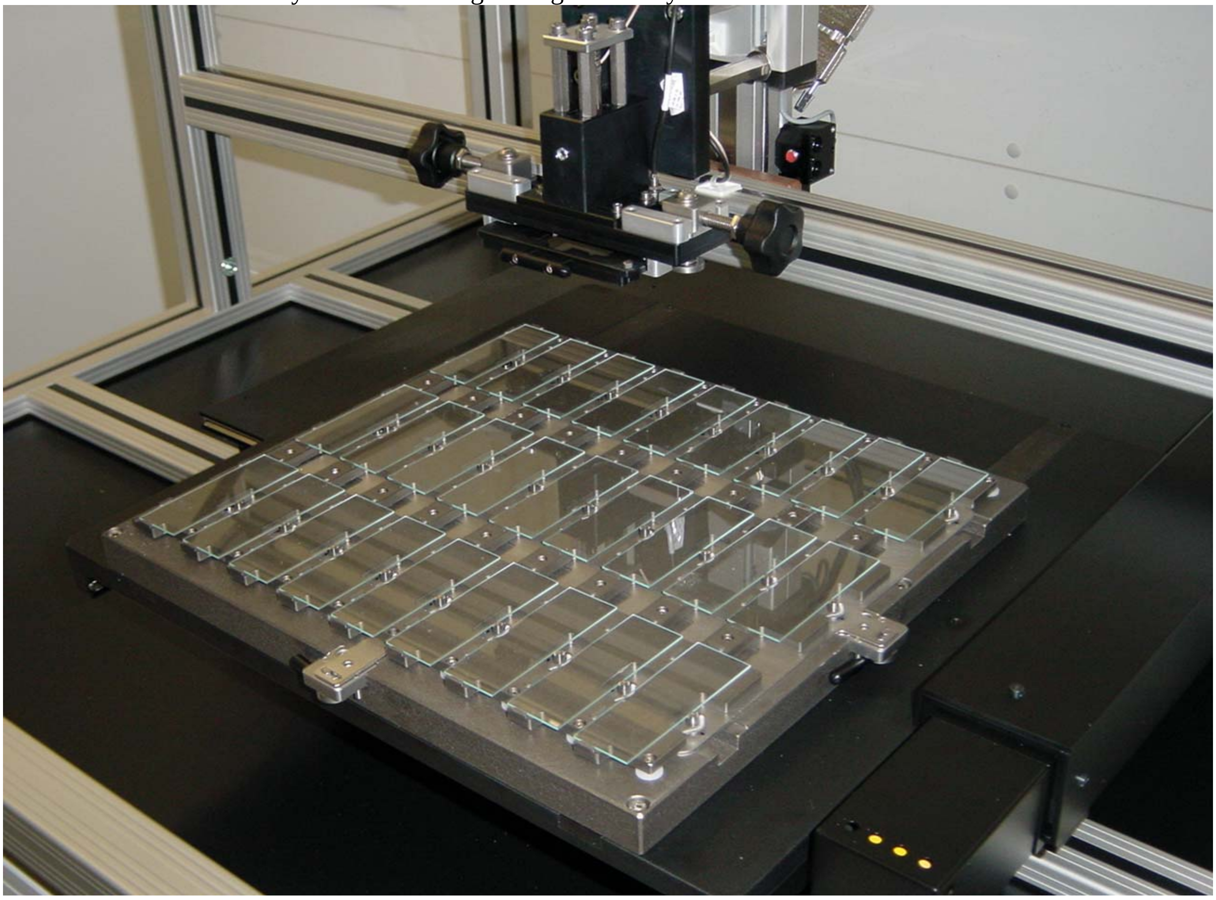

Fig. 7: Glass plate arrangement

The LANG LSTEP-PCI positioning system is a high-resolution stepping motor controller in the form of a PC plug-in module for the PC. This device is used to control three axes with 2/4 phase stepping motors.

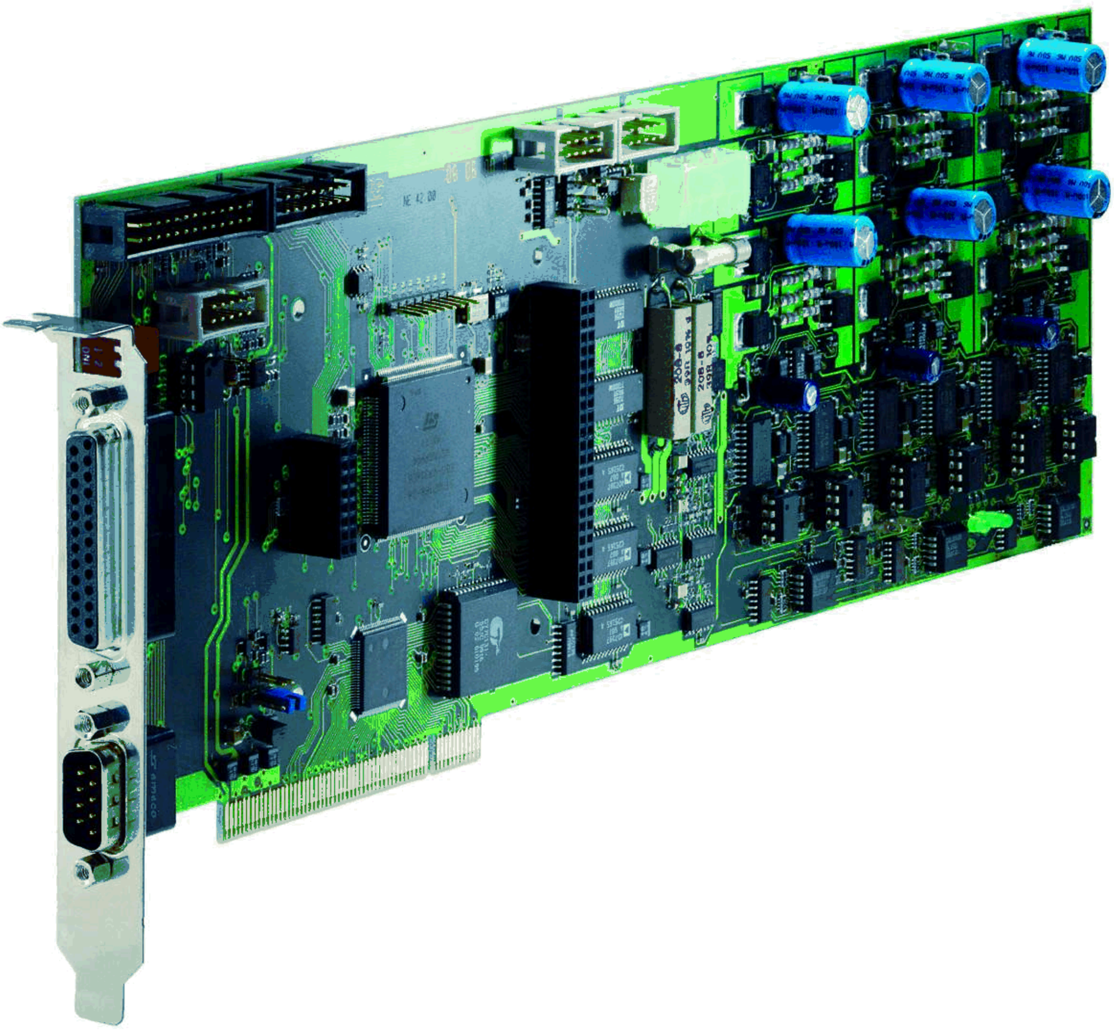

Fig. 8: LANG LSTEP-PCI positioning system

Communication between the PC and the controller takes place through the PCI bus topped by a Dual-Port Ram. Dynamic micro step operations allow positioning operations to be done quickly and with the highest precision.
The integration of a controller and power amplifiers on a plug-in device for the PC provides compact, EMC-compliant systems without additional mechanical requirements. This solution is ideal for applications where cost is a sensitive factor. A wide range of equipment options provides more flexibility, allowing to adapt the system to meet individual needs.
The price performance ratio is excellent because of the lack of an external chassis with implemented power amplifiers.

Software

LabVIEW is the ideal platform for integrating different Virtual Instruments in one application. LabVIEW 7 Express marks a new milestone in Graphical Dataflow Programming. The TopSpot software utilizes new features of LabVIEW 7 Express, especially dynamic event management.
Together with a message based and event controlled state machine the TopSpot application only uses some percent CPU power. The hole application has NO local variables. The DAQ devices are controlled using the traditional DAQ library (the new DAQmx environment does not support all NI hardware components used by now).
The system is extremely robust because the WindowsXP explorer shell is replaced by the TopSpot application. System procedures that are not required are not started.

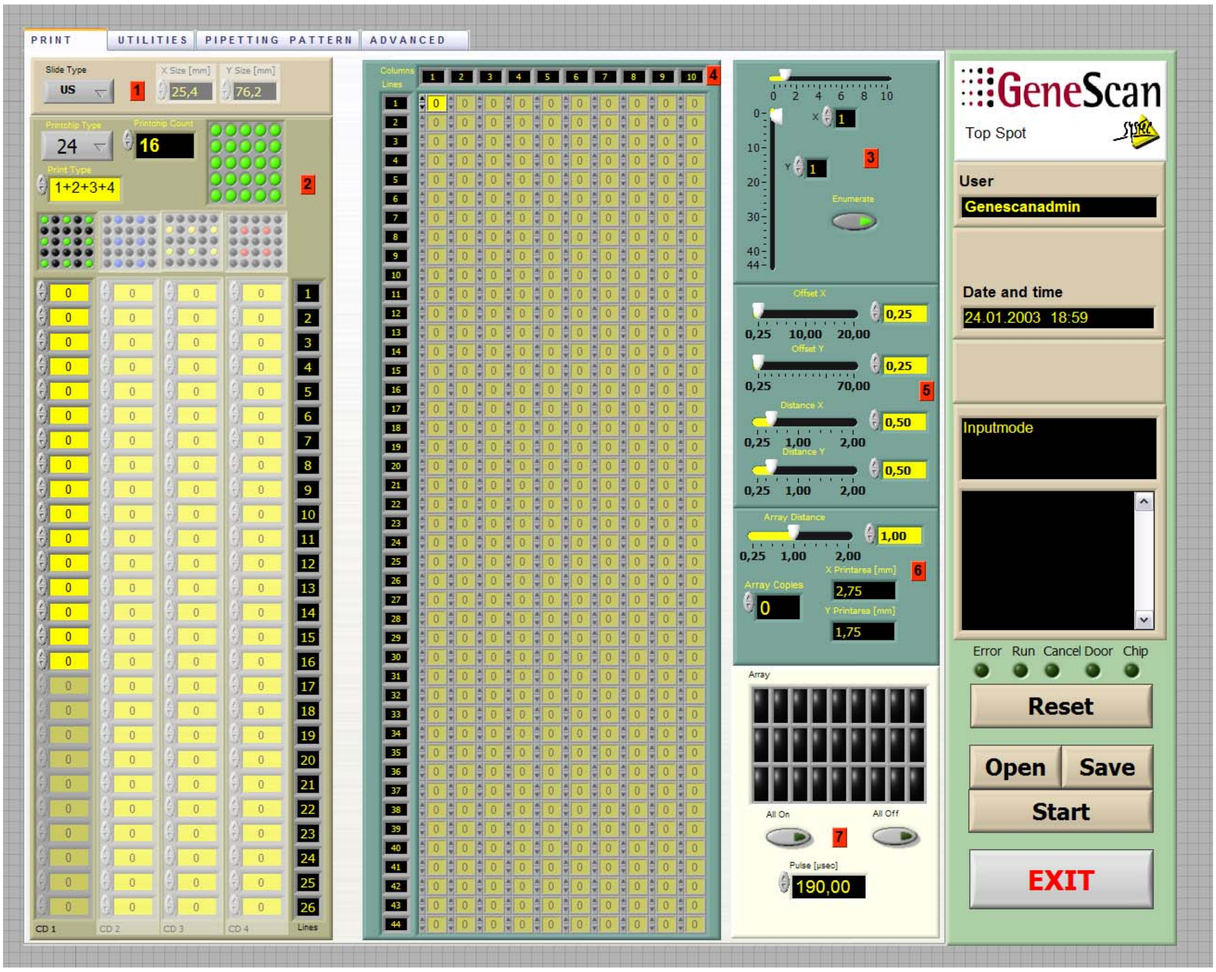

fig. 9: Front panel

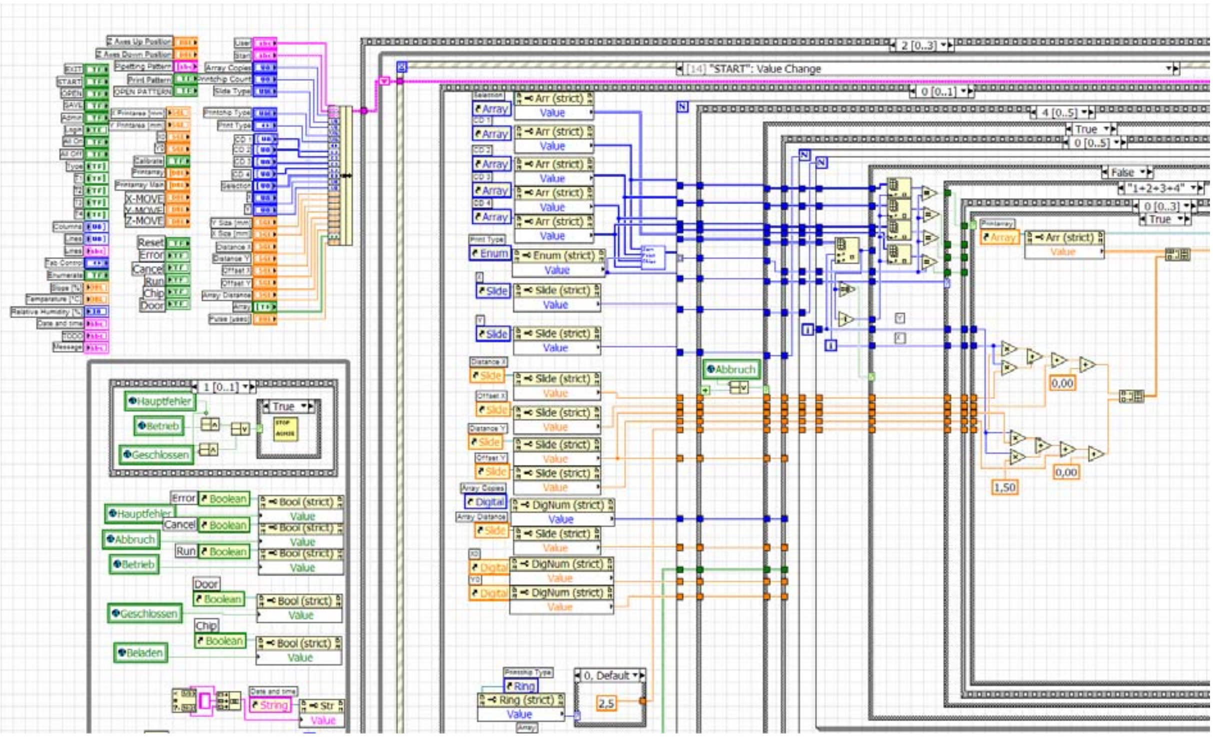

fig. 10 Part of block diagram (main VI)

The user interface was developed using sophisticated SYSTEC ergonomic guidelines. All relevant informations are visible on one tab control instance. 70 percent of the VI´s take care of plausibility checks and error management. The Webworks help subsystem uses SYSTEC´s unique Video To Web software introducing unrivalled text to speech technologies. The user interface supports multiple languages.

It is essential to define software standards for each project. As TopSpot is a product that is distributed worldwide the quality of the software and hardware components is in the center of interest.
The TopSpot software development plan is based on a waterfall development model. The waterfall model is the classic model of software engineering. It has deficiencies, but it serves as a baseline for many other lifecycle models. The pure waterfall lifecycle consists of several non-overlapping stages. It begins with the software concept and continues through requirements analysis, architectural design, detailed design, coding, testing, and maintenance.

The software requirement standards (SRS) are based on GPP (good programming practice) standards of the LabVIEW Usergroup Central Europe e.V. (internal Draft).
Basic thoughts of Grady´s and Caswell´s FURPS (Functionality, Usability, Reliability, Performance, and Supportabilty) model [6] are implemented in this model.
Metrics are defined to deal with following FURPS items:

- Functionality
    - Feature set
    - Capabilities
    - Generality
    - Security

- Usability
    - Human factors
    - Aesthetics
    - Consistency
    - Documentation
- Reliability
    - Frequency/severity of failure
    - Recoverability
    - Predictability
    - Accuracy
    - Mean time of failure
- Performance
    - Speed
    - Efficiency
    - Resource consumption
    - Throughput
    - Response time
- Supportability/Maintainability
    - Testability
    - Extensibility
    - Adaptability
    - Compatibility
    - Servicability
    - Installability
    - Localizability

Conclusion

TopSpot D is a LabVIEW based contact-free printing system for biomolecules. The system is easy to use, extremely cost effective, and very fast. Incorporating the latest hard and software technologies leads to unrivalled productivity.

The author

Herbert Pichlik was born in 1958; he studied electrical engineering at the Georg-Simon-Ohm University of Applied Sciences in Nuremberg. He started his professional career in 1985 when he joined Philips Kommunikations Industrie AG (PKI) as a software development engineer. Later, he moved to the quality management department at PKI. After a short period at LGA, he joined Quelle AG in 1990, where he has been in charge of measuring and test instrument management as well as test instrument development. After being product manager at Testware he joined SYSTEC GmbH in mid 2000 as manager of the test and automation division.
Herbert Pichlik has written and coauthored several books and dozens of papers and articles. Since 1992, when he assumed responsibility for a large number of different projects, he has worked intensively with LabVIEW. Herbert Pichlik is an internationally awarded synergist, enthusiastic squash player, father of four children, and owner of several patents in the field of analog and digital integrated circuit technologies; he started lecturing in graphical data flow programming at the Nuremberg University as a sideline in 1997. Since 2002 he is president of the LabVIEW Usergroup Central Europe e.V. (www.lvug.de)

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