Design of geniom, a novel and fully integrated benchtop array facility

Geniom (figure 1) is a novel microarray platform that integrates all functions needed to perform any array-based experiment in a compact instrument on the researcher´s laboratory benchtop. The system supports light-activated *in situ* synthesis of oligonucleotide probes, array hybridization, and CCD-based array detection. The essential components of the instrument include the optical unit for synthesis and detection, a mobile tempering element for temperature control during the hybridization process, a fluidic system delivering all reagents required for array synthesis and hybridization from storage containers to the reaction carrier, and an internal computer controlling all functions of the device. The internal computer is connected to another external computer running the software that supports all steps from array design to data analysis.

Figure 1
The geniom instrument (exterior)

Along with the geniom device we developed a new type of reaction carrier (DNA processor). Inside this carrier, oligonucleotide probes are synthesized in three-dimensional channels instead of a planar microscope slide. The following section will describe the reaction carrier as well as the main components of the instrument in more detail.

The reaction carrier (DNA processor)

The DNA processor (figure 2A, B) is a three-dimensional, micro-machined reaction carrier, composed of a structured 100 µm silicone layer enclosed in a glass cover sandwich. The overall size of the reaction carrier is approximately 2.1 cm x 1.9 cm.

The silicone structure consists of 56 channels, each 220 µm wide. Up to 1,000 features of 33 µm x 33 µm at a regular spacing of 18 µm can be placed into one channel. Groups of channels are interconnected, providing a number of individual sub-chambers (referred to as arrays). The reaction carrier used in our study features four arrays, each combining fourteen channels in a meandering system (4 arrays carrying 14,000 features each). The upper glass cover bears several perforations that connect the channel systems in the silicone layer to the fluidic system of the geniom device. Thus, a single reaction carrier contains 4 individually addressable arrays, each with its own inlet and outlet and an inner volume of approximately 5 µl.

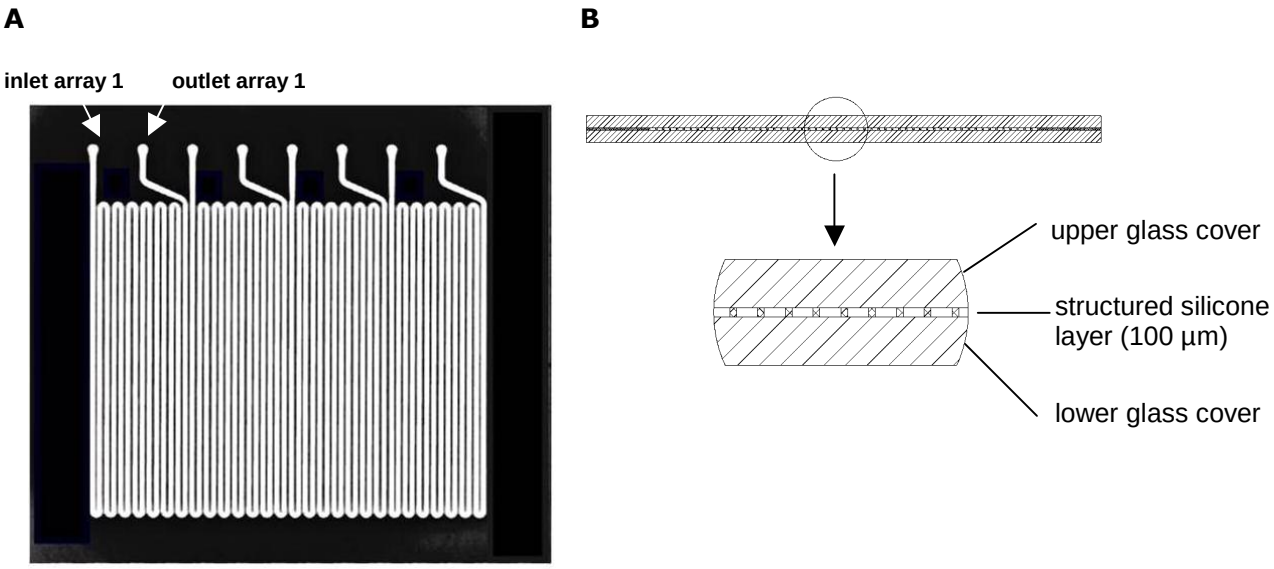

Figure 2: Schematic representation of the reaction carrier

(A) Reaction carrier with 4 individual arrays. In each array up to 14,000 features (33 μm^2 area; 18 µm spacing) can be synthesized. **(B)** Cross-section of the reaction carrier. The reaction carrier consists of a structured silicone layer covered by two glass layers.

For oligonucleotide synthesis, the reaction carrier must be initialized by a surface treatment which includes activation of the glass surface and addition of molecular spacers that will accept the oligonucleotide probes.

To connect the reaction carrier to the geniom system, it is first placed in a special cartridge. The cartridge slides into the instrument pressing the DNA processor firmly

against a register plate. Simultaneously, the reaction carrier is connected to the so called "micro-macro interface". This interface connects the microscopic arrays (via their inlets and outlets) to the macroscopic tubing of the fluidic system. The design of the "micro-macro interface" allows to address each array individually. Once adjusted in the device, the reaction carrier can be heated or cooled to any temperature between 20°C and 90°C by a Peltier tempering module that automatically contacts the surface of the reaction carrier (for example during hybridization).

The fluidic system of the geniom device

During *in situ* oligonucleotide probe synthesis as well as hybridization, the channel-like arrays are flushed with various reagents, samples, or washing buffers. This is achieved by an elaborate fluidic system (figure 3). Delivering the appropriate reagent at the right time to the right array is essential to all functions of the device. These critical steps are therefore carefully controlled by software running on the integrated computer. Two multiple-valve blocks form the heart of the fluidic system. The ports of the first block (synthesis block) are connected to the reservoirs of the reagents needed for oligonucleotide synthesis, while the ports of the second block (hybridization block) are connected to the reservoirs of the hybridization solutions. Using argon as an inert gas, each reservoir is set under controlled pressure that serves as the only moving force for all solutions in the system. The argon also provides a protective atmosphere for the highly sensitive synthesis chemicals. The inlet of each array of the reaction carrier is connected to one port on the synthesis block, while the outlet of each array is connected to one port in the hybridization block. In addition, one port of each block is connected to the waste path. Reservoirs, multiple valve blocks and the micro-macro interface are connected by 1/16” teflon tubing.

To flush one array with a certain synthesis chemical, the valve of the specific chemical reservoir in the synthesis block, the two valves of the desired array and the valve of the waste path in the hybridization block are opened. The pressure gradient between the chemical reservoir and the waste path pushes the solution through the addressed array (figure 3). Hybridization solutions are flushed through the arrays in the same way.

In the waste path, the solution passes two optical elements to measure the volume flow. At the end of the waste path, the solution enters a waste reservoir.

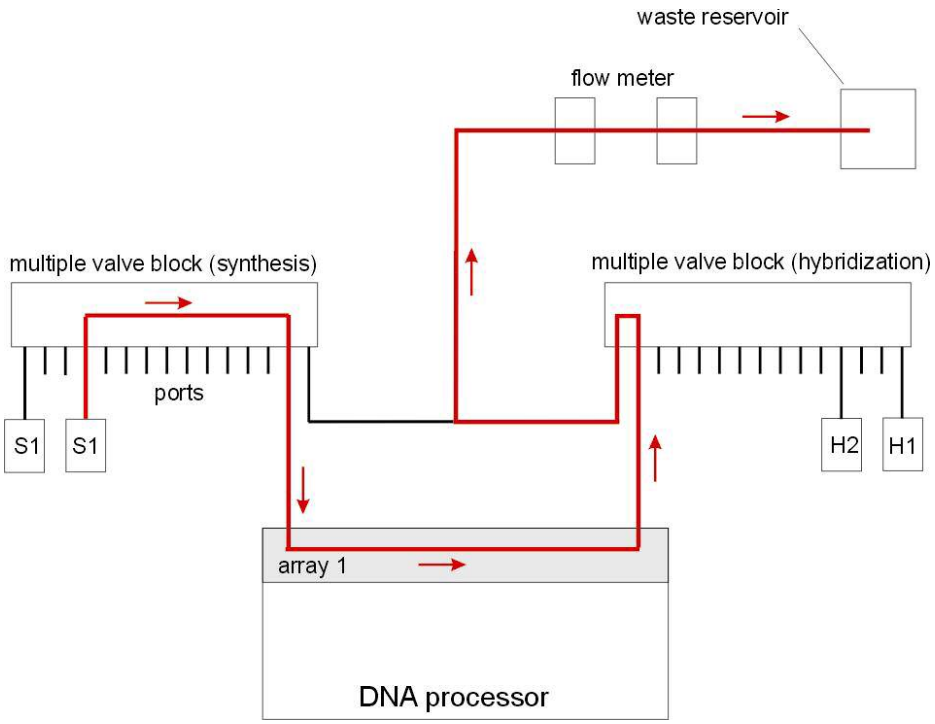

Figure 3: Schematic representation of the fluidic unit in the geniom device

The image exemplifies how array 1 is flushed with a certain reagent needed during *in situ* oligonucleotide synthesis. For flushing with the reagent [S1], the [S1] reservoir is set under pressure. Simultaneously, 2 ports in the synthesis block and two ports in the hybridization block are opened. In the synthesis block, the port of reservoir [S1] and the port to array 1 are opened. In the hybridization block the array 1 port and the to the waste port are opened. The pressure gradient between the [S1] reservoir and the waste container results in a flow of [S1] through array 1 into the waste. The flow is monitored by means of the flow meter.

The geniom optical unit

The optical unit is one of the central components of the geniom instrument. It is essential for light-activated *in situ* synthesis of oligonucleotide probes as well as exciting and detecting fluorescence signals.

When the reaction carrier slides in position and contacts the "micro-macro interface", it is simultaneously adjusted in the optical path (figure 4).

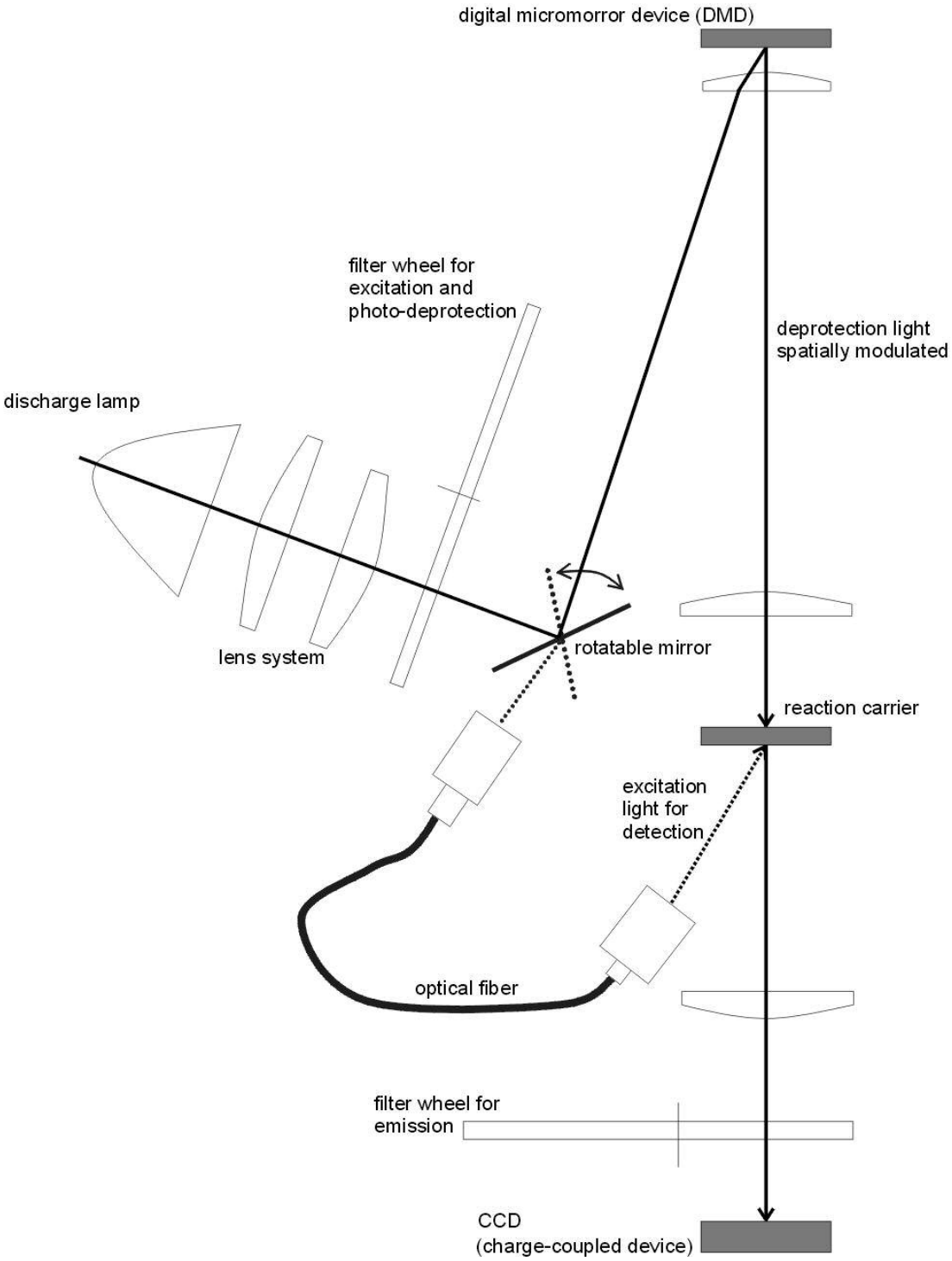

Figure 4: Schematic view of the optical unit

In the optical system, a short-arc discharge lamp emits light with a continuous spectral range of 270 nm to >800 nm. After passing a lens system, the UV light in the range of 350 nm to 440 nm is modulated and projected on the DNA processor for photo-deprotection of the phosphoramidites during synthesis.

The core element of the optics unit, the digital micro-mirror device (DMD, Texas Instruments, Inc.), is a spatial light modulator. A very similar device is also used in beamers for digital video projection. The DMD contains an array of 786,432 hinge-mounted microscopic mirrors. Each of these computer-controlled mirrors measures approximately 16 µm x 16 µm. The tiny hinges enable each singular mirror to tilt either toward the light source or away from it, creating a light or a dark pixel on the array. Due to an imaging scale of 1:1, the projection optics generate pixels of the same size inside the DNA processor. As a current standard, 2 x 2 DMD pixels are clustered to create a single features (33 μm^2 in size).

For fluorescence detection with the integrated CCD (charge-coupled device), a rotating mirror reflects the light into a optical fibre. Dark-field illumination is used to create a uniform excitation of the fluorescent dye across the DNA processor. To provide the narrow spectral ranges required for deprotection and excitation, several filters can be positioned in the optical path using a filter wheel. Another wheel located in front of the CCD contains appropriate emission filters. Both filter wheels contain several filter sets for different fluorescent dyes, including Cy5 and Cy3. The CCD consists of 3500 by 2300 pixels of 10 µm x 10 µm each without gaps between the pixels. Thus, each feature of 2 by 2 DMD pixels is read out by approximately 30 CCD elements. The optics for fluorescence detection are corrected for wavelengths above 400 nm. Consequently, the CCD may not only be used for fluorescence detection but also to monitor the UV light intensity during photo-deprotection. This allows real-time quality control of the photodeprotection step.

Light-activated *in situ* synthesis of oligonucleotide probes

During light-activated oligonucleotide probe synthesis, coordinated function of all main units of the geniom device is required.

The oligonucleotide synthesis uses standard reagents and phosphoramidites with a photolabile 5´-NPPOC (2-[2-nitrophenyl]-propoxycarbonyl) protecting group. As a first step, a linker molecule with a NPPOC protecting group is added to the tips of the spacer molecules, thereby activating the array. Probe synthesis is done in parallel on all arrays of one reaction carrier. The synthesized probe sets may be the same or different for all 4 arrays. Once the desired probe sets have been chosen (or

designed), the geniom software takes over and automatically runs the complete synthesis process. The sequence of coupling steps required to synthesize the desired probes is calculated. Then probes are synthesized step by step in a cycling process (figure 5).

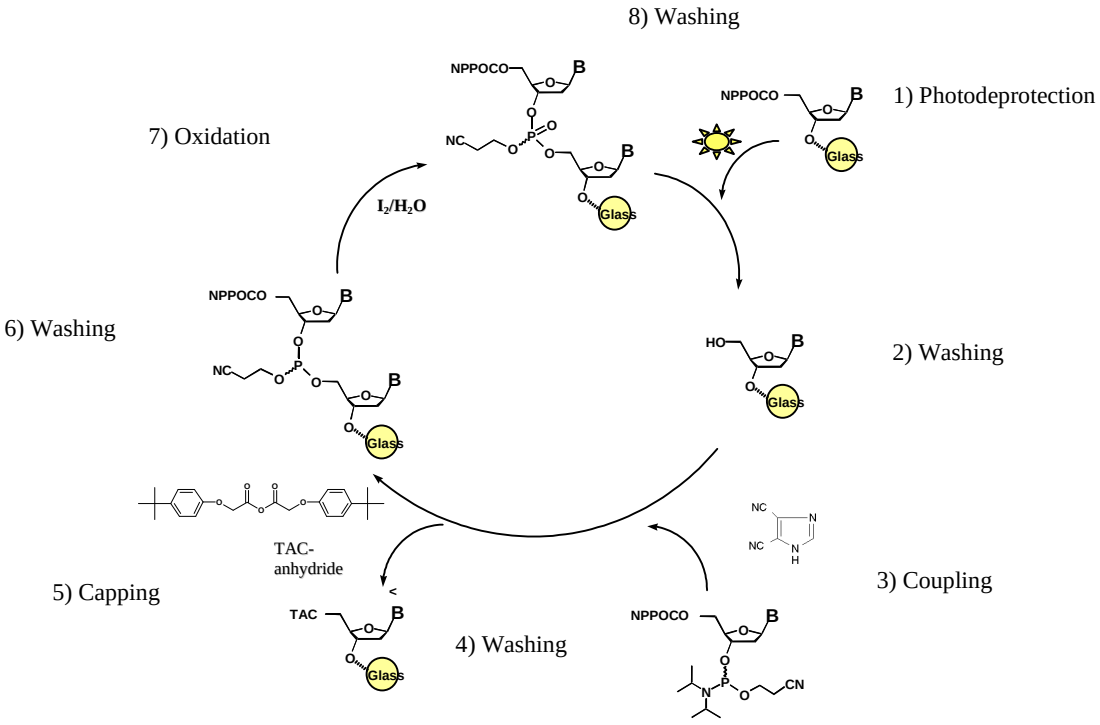

Figure 5: Elongation of oligonucleotide probes during *in situ* synthesis

Different oligonucleotides can be synthesized on one array via light-activated synthesis. Only the oligonucleotides on the spots exposed to light will be elongated during the next cycle. All other 5'-OH groups remain protected by the photolabile protecting group and can be elongated with a different amidite during the next cycle.

The time needed for the synthesis of standard arrays is independent of the number of different probe sets, the sequences of the probes synthesized and the number of different probes within one probe set. However, it is strongly influenced by the probe length. Straighforward step-by-step oligonucleotide synthesis may be achieved by flushing the arrays with the appropriate reagents and exposing individual features on the arrays to light. This procedure requires 4 times n cycles (where n is the length of the oligonucleotide probes). In contrast, the geniom software allows the oligonucleotide probes to have a different lengths during synthesis and calculates an optimized sequence of coupling steps. This results in a smaller number of cycles

required for the complete synthesis. For instance, the synthesis of 25mer probes only requires 83 cycles instead of 100. According to the conservative protocol used in our study (ref.), one cycle takes approximately 9.5 min. Thus, the synthesis of 25mer probe sets in all four arrays on one reaction carrier takes approximately 15.5 h (83 x 9.5 min ≈ 13 h + 1.5 h for the final deprotection of the purine and pyrimidine bases). With the updated protocol currently in use, the synthesis of four 25mer arrays is achieved in approximately 8.5 h (83 x 5 min ≈ 7 h + 1.5 h for the final deprotection).

In contrast to the synthesis which is always done in parallel on all four arrays of one reaction carrier, the hybridization is more flexible and can be done either in parallel or sequentially.