BIOASSEMBLER

Integrating bio-inspired assembly into semiconductor manufacturing technology for biosensors

D4.3 Illumination system for wafer-scale DNA synthesis

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Revision History

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List of contents

List of Figures

1. Background and objectives

A central element of DNA photolithographic synthesis is the photodeprotection reaction, where light is used to remove protecting groups from the 5' hydroxyl (OH) ends of the DNA strands. These protecting groups, which prevent unwanted reactions during the synthesis process, are selectively deprotected by exposure to UV light, generally 365 nm. When the UV light shines on specific regions of the surface, it deprotects the 5' OH groups, making them available for the addition of nucleotides in a controlled manner. This stepwise process of deprotection and nucleotide incorporation allows for the precise assembly of desired DNA sequences at designated locations on the surface.

A significant challenge in the BIOASSEMBLER project is scaling up the optical system for photolithographic DNA synthesis to accommodate wafer-scale production. This requires an optical system with the capability for spatial control to illuminate specific positions on the wafer's surface. Additionally, the system must enable the simultaneous or rapid successive illumination of thousands of positions on the surface. Maskless approaches are particularly advantageous, as they reduce costs and increase synthesis speed by eliminating the need for physical masks to define light exposure areas. Among the various methods available for maskless light delivery, the use of a UV laser scanning system stands out due to its high-intensity light source. This system offers several advantages, including shorter exposure times and increased throughput.

In this deliverable, we detail the development of a prototype laser scanning system for the photolithographic synthesis of DNA. The prototype device, built by VTT, consists of a 405 nm laser, a scanning mirror, and programmable controls for laser pulse position, power, and exposure time. The device was designed to be tested in *slide scale* DNA synthesis at 405 nm using phosphoramidites with the 5' SPh-NPPOC protecting group, a nitrophenylpropyloxycarbonyl (NPPOC) derivative. The fundamental photochemistry of the SPh-NPPOC group is similar to that of NPPOC or the widely used Bz-NPPOC. However, it has been reported to have improved photocleavage efficiency, allowing for faster syntheses using 365 nm UV light.[1] Another characteristic of SPh-NPPOC is its absorption at wavelengths up to 420 nm (Figure 1). Despite there being no previous reports, we hypothesized that this feature could make it compatible with photodeprotection reactions using light sources within these wavelengths. Performing synthesis at 405 nm offers significant advantages, as (i) lasers at this wavelength are generally less expensive compared to those emitting at 365 nm, (ii) many polymer-based flow cell materials become optically transparent and (iii) DNA is less prone to degradation at 405 nm, which could result in higher quality synthesized DNA sequences.[2]

Herein, we discuss the technical aspects of the prototype system, including its design and operational parameters, as well as the main features of the 405 nm laser-assisted DNA synthesis tested at UniVie.

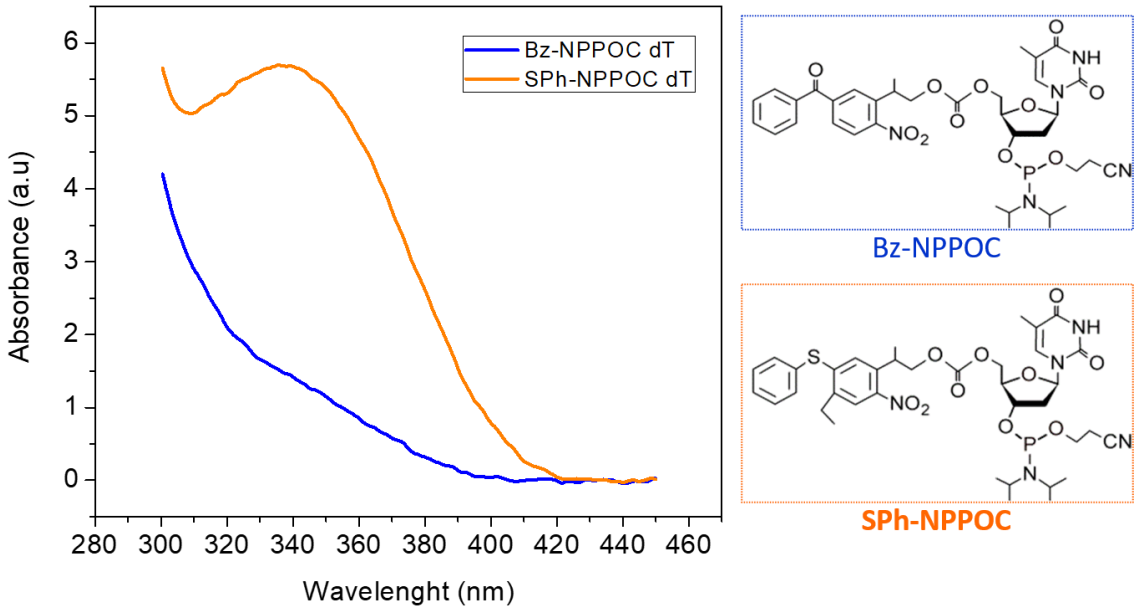


Figure 1 – Absorption spectra of Bz-NPPOC and SPh-NPPOC DNA-T phosphoramidites in DMSO along with the respective structures.

2. BIOASSEMBLER laser exposure system: design and technical aspects

A custom laser scanning system was built to study the feasibility of short intense pulses of light in DNA zipcoding. The important requirement was the ability to control illumination intensity over several orders of magnitude. The realized system enables flexible combination of a wide range of irradiance settings (W/cm^2) with different durations of illumination to achieve a desired total radiant exposure (J/cm^2).

Figure 2 presents a schematic of the laser scanner, and Table 1 summarizes the system's technical properties. The system was assembled around a fast-steering mirror (Optotune MR-15-30) and 100 mW 405 nm laser (Roithner Lasertechnik RLDE405M-100-5). The 405 nm wavelength was chosen due to the availability of low-cost laser light sources and optics compared to cost of parts in the ultraviolet range, and with the expectation that the SPh-NPPOC protecting group could be photolyzed even at this wavelength (see Figure 1 and discussion in the Background section).

A custom power attenuation system was created using a combination of two polarizers and a half-wave plate. This combination can achieve five orders of magnitude power attenuation and could be adjusted to another wavelength by replacing only the waveplate. Figure 3 shows the complete laser system with and without the safety enclosure. The enclosure includes an aperture for laser exit with a protective cap.

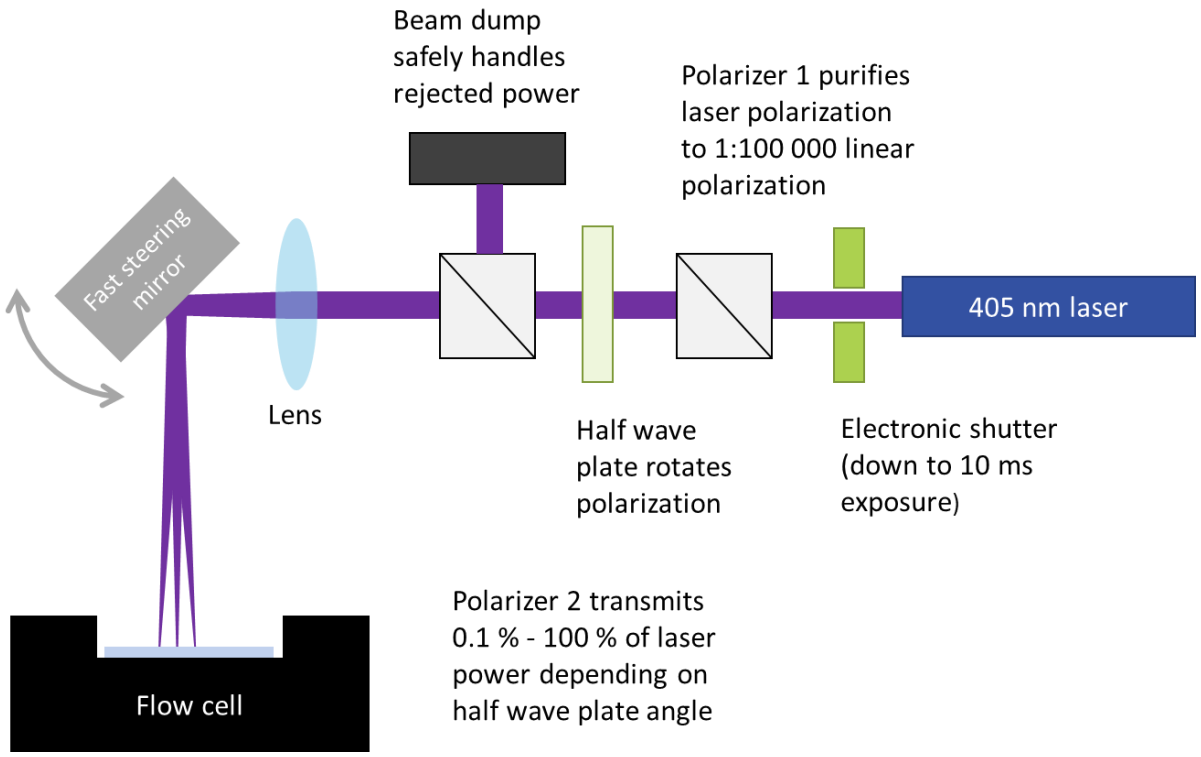


Figure 2 - Schematic of the laser scanner

Table 1 - Technical properties of the laser scanning system

Laser wavelength after 10 min warmup	404.2 ± 0.5 nm
Laser power	112 mW
Power at target	0.04 – 75 mW
Power accuracy	<10 % based on device specification for 0.15-75 mW <3 % tested for 0.1 %, 1 %, 10 % and 100 % settings
Laser pulse length accuracy	1-4 ms
Scanning mirror stabilization time	3-5 ms manufacturer specification
Pointing stability	<40 µm position variation at target over 1 minute
Spot size at focus	<50 µm, notably elliptical

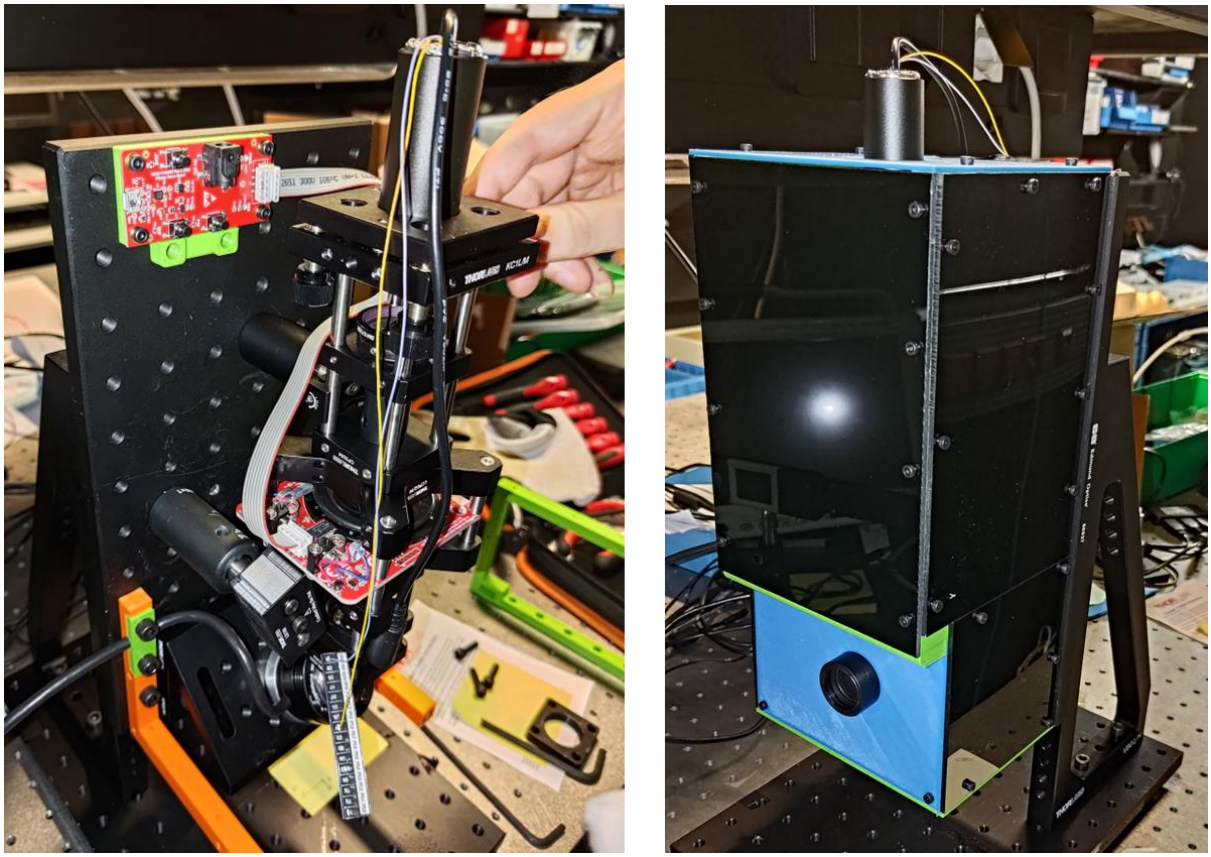


Figure 3 - Photographs of the laser scanner without and with laser safety enclosure.

Figure 4 corresponds to a beam camera image showing the beam profile out of focus. The beam exhibits a roughly elliptical shape with intensities concentrated in the central area. Diffraction effects due to the use of a 5 mm diameter polarizer are also observed. The beam diameter in focus was estimated to be around 50 µm. However, this and other characteristics of the laser spot in focus could not be accurately measured due to the insufficient resolution of the camera (Figure 5).

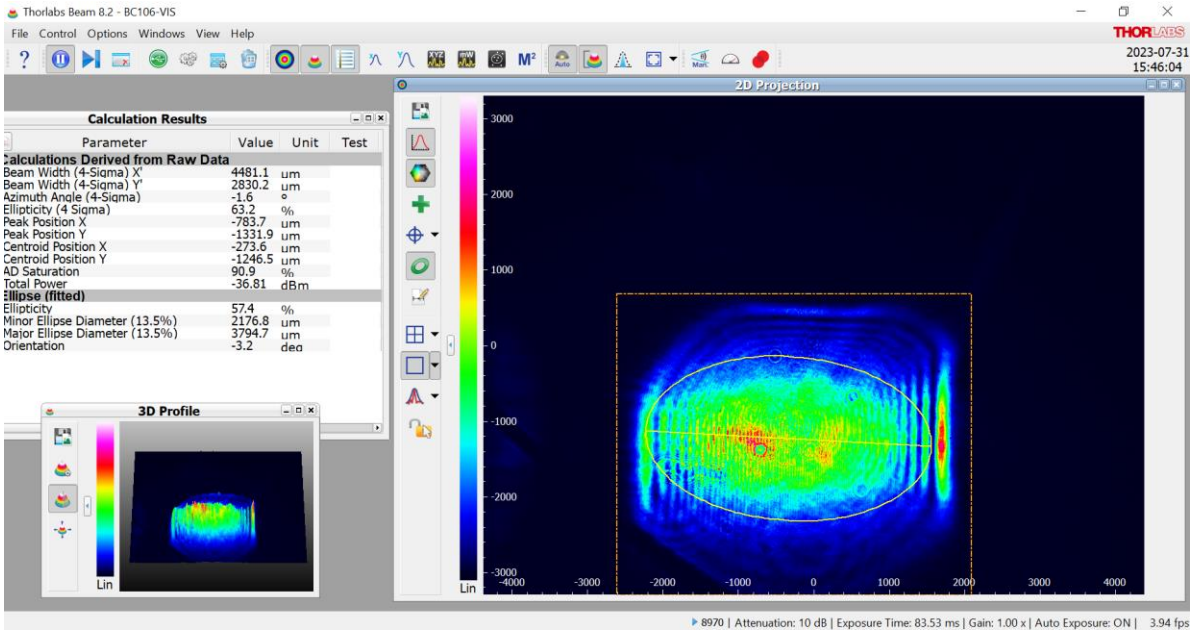


Figure 4 - Beam camera image showing the ellipticity of the laser beam and diffraction effects caused by 5 mm diameter polarizer.

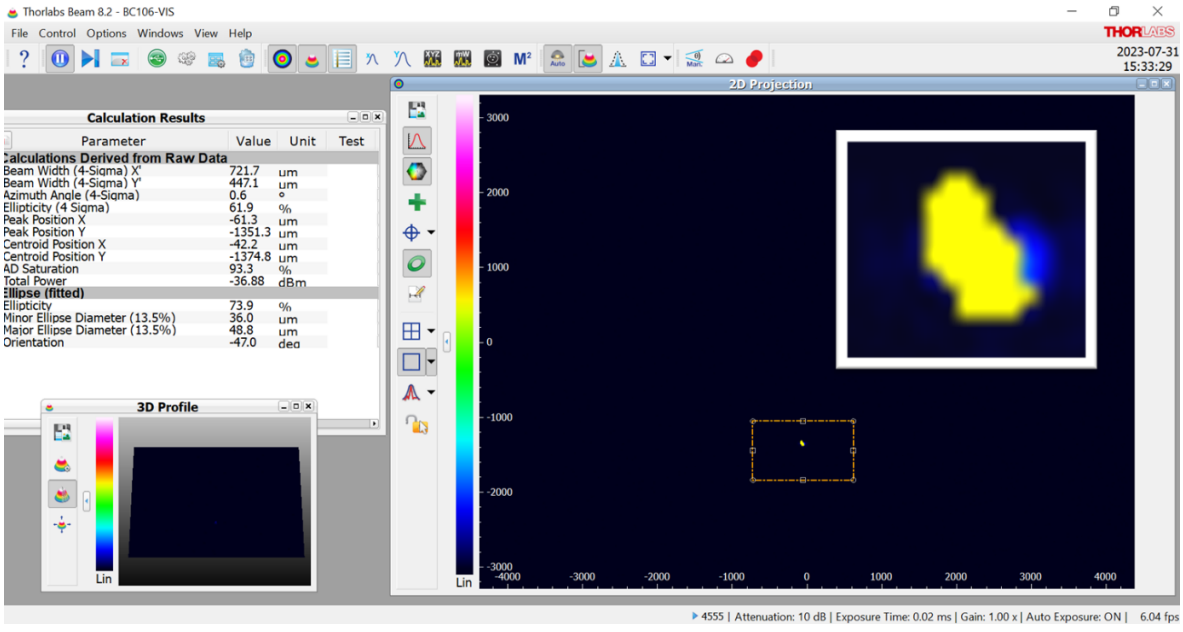


Figure 5 - Beam camera image of the laser spot in focus. Due to 5 µm pixel size of the camera, beam characteristics cannot be very accurately determined.

For operation, the laser scanner is connected to a computer via a USB connection. A LabVIEW program was developed to allow for controlling parameters such as illumination position, time, and laser power. To achieve the desired laser power, the power level is set as a percentage of the maximum power, and the laser scanner software calculates the necessary waveplate angle based on experimentally obtained calibration data (Figure 6).

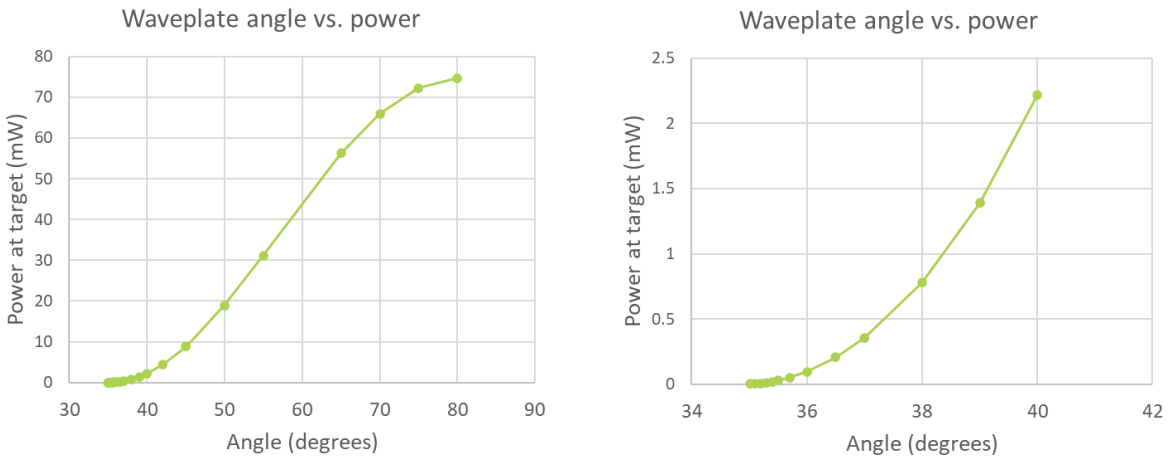
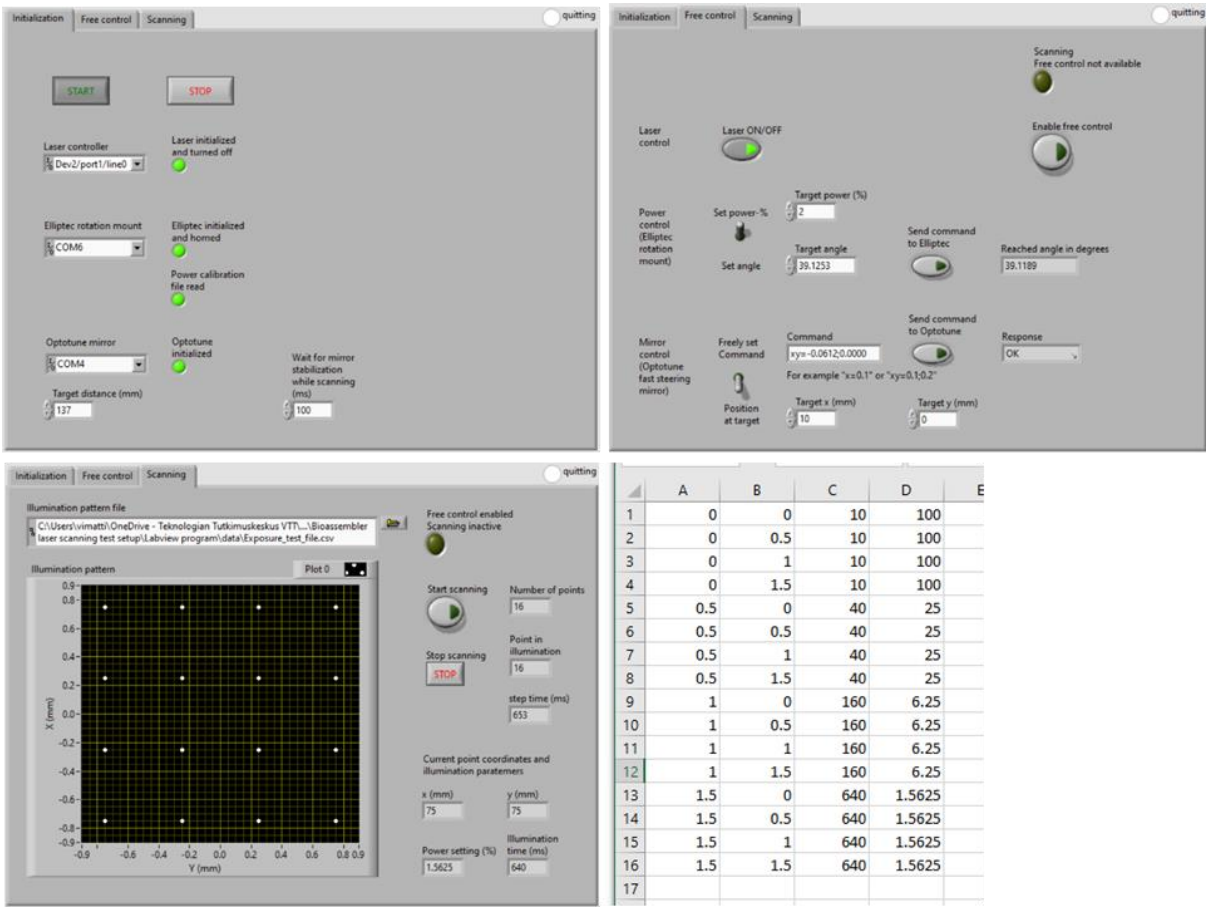


Figure 6 - Data used for power calibration. Laser scanner software calculates the required waveplate angle using the calibrated data when desired power level in percentage of maximum power is given.

The LabVIEW program interface, shown in Figure 7, features an initialization tab and a free control tab that enables sending commands to the laser controller, rotation mount, and power controller. Automatic scanning of a number of points can be performed using the scanning tab. The point pattern to be scanned is provided in a CSV file with four columns: (1) positioning in X (in millimeters), (2) positioning in Y (in millimeters), (3) laser pulse time (in milliseconds) and (4) laser power setting (in percentages). An example file is also displayed in Figure 7.



	A	B	C	D	E
1	0	0	10	100	
2	0	0.5	10	100	
3	0	1	10	100	
4	0	1.5	10	100	
5	0.5	0	40	25	
6	0.5	0.5	40	25	
7	0.5	1	40	25	
8	0.5	1.5	40	25	
9	1	0	160	6.25	
10	1	0.5	160	6.25	
11	1	1	160	6.25	
12	1	1.5	160	6.25	
13	1.5	0	640	1.5625	
14	1.5	0.5	640	1.5625	
15	1.5	1	640	1.5625	
16	1.5	1.5	640	1.5625	
17					

Figure 7 - LabVIEW user interface and test exposure file with position in x (mm), position in y (mm), exposure time (ms) and laser power (%) as the columns.

3. Laser assisted DNA photolithographic synthesis

The fully assembled system for DNA synthesis at 405 nm is shown in Figure 8. This system consists of the laser device, the reaction cell used to position the synthesis surface (glass slides) at the exact focal point of the laser, and the fluidics system used to deliver all the chemicals and solvents necessary for the synthesis. Both the laser device and the cell holder were assembled on an optical breadboard. For the fluidics system, an Expedite 8909 nucleic acid synthesizer was used. The focal point for positioning the cell was determined by assembling the cell and placing radiochromic film between glass slides. The film was then exposed to the laser, and the position of the cell was adjusted until elliptical spots with minimal blurriness were obtained.

The tests performed with the laser system discussed herein aimed at evaluating the possibility of performing DNA synthesis using a 405 nm light source. To do so, a 25-mer DNA oligonucleotide on a

T5 linker was synthesized on functionalized glass slides following protocols for functionalization and synthesis previously optimized for photolithographic synthesis at 365 nm.[3] The exposure solvent, which is in contact with the surface during light exposure, was a 1% (w/v) solution of imidazole in DMSO. All the experiments were done by introducing a β-carotene solution in the back chamber of the synthesis cell, to prevent UV light reflection from the quartz block back to the cell.[4] After deprotection, the arrays were hybridized with Cy3-labeled complementary strands.

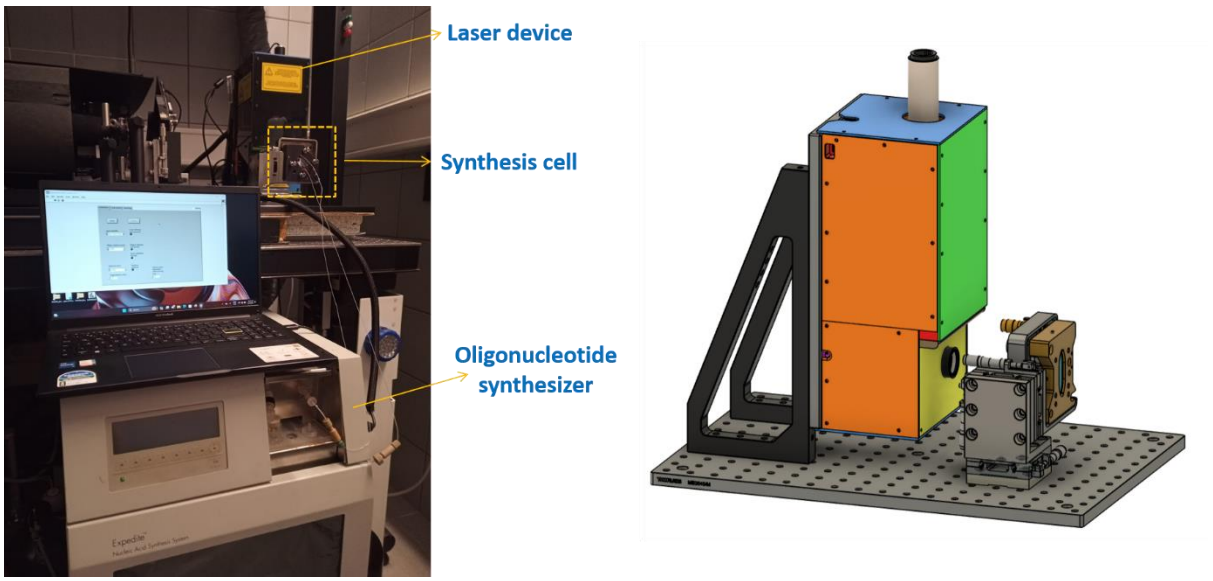


Figure 8 - Photograph of the fully assembled system for laser-based DNA synthesis along with a scheme of the optical system assembled on a breadboard. The fluidics delivery, an Expedite 8909 nucleic acid synthesizer, is connected to the fluidic cell positioned at the focal point of the laser. Also visible is the control computer which is connected to the laser system.

When using 365 nm UV light, 0.5 J/cm² exposures are necessary to remove 95% of SPh-NPPOC protecting groups, which is approximately 12 and 6 times less than required for NPPOC and Bz-NPPOC protecting groups, respectively. This improvement has been attributed, in part, to the high absorption of SPh-NPPOC in the spectral region near 365 nm.[1] Based on the absorption spectra in Figure 1, one could expect photolytic efficiencies for SPh-NPPOC at 405 nm to be close to those observed for Bz-NPPOC at 365 nm. However, since we did not have information about how using fast exposure times (in the range of milliseconds) could affect the photolysis reaction, different synthesis conditions were tested.

Figure 9 shows the scans and the average fluorescence intensity for features synthesized using three different radiant exposures: 6, 30, and 100 J/cm². Synthesis using different laser powers was also conducted to evaluate the effect of exposure time on signal intensities. The radiant exposure is calculated as the product of the irradiance (W/cm²) and the exposure time. In our setup, irradiance is determined by the laser power and the area over which the laser beam is spread. To obtain accurate radiant exposure values, it is essential to know the size of the laser beam spot on the synthesis surface. Even though the laser beam diameter estimated using a bean camera was around 50 µm, during both the synthesis process and measurements conducted with radiochromic film, we observed a wide range of spot sizes that often exceeded the expected 50 µm diameter. Given this variability and the lack of a precise method to determine the actual irradiated area, we decided to use a fixed laser beam size of 100 µm for our calculations to maintain consistency.

The fluorescence data was extracted from the central area of the features where higher beam intensity is expected. The low-cost laser used in the prototype device has a roughly elliptical beam profile lacking a well-defined intensity distribution (Figure 4). The peripheral areas of the spot receive less light than the center, but will become visible in overexposure conditions. Using high radiant exposures resulted in enlarged spots with only modest increments in fluorescence intensities in the central areas, indicating saturation in the center while the peripheral areas still benefited from increased exposure. The irregularities in the spot shape are partly explained by the suboptimal laser beam profile and partly by the optics, but they may also be attributed to reflections of light inside the synthesis cell, which promote unintended deprotection reactions when excessive exposure times or irradiances are used. These results suggest that the laser-assisted synthesis of DNA does not require significantly higher radiant exposures, and that efficient deprotection may be achieved using light doses closer to those required for synthesis with Bz-NPPOC protecting groups at 365 nm. This was verified by synthesizing the same 25-mer sequence using a gradient of light exposures ranging from 0.1 to 12 J/cm². As the exposure increases, the fidelity of the synthesized sequences improves until the full hybridization signal is reached. Figure 10 shows the photolysis curve, where it can be seen that the plateau signal is already obtained for exposures as low as 1 J/cm².

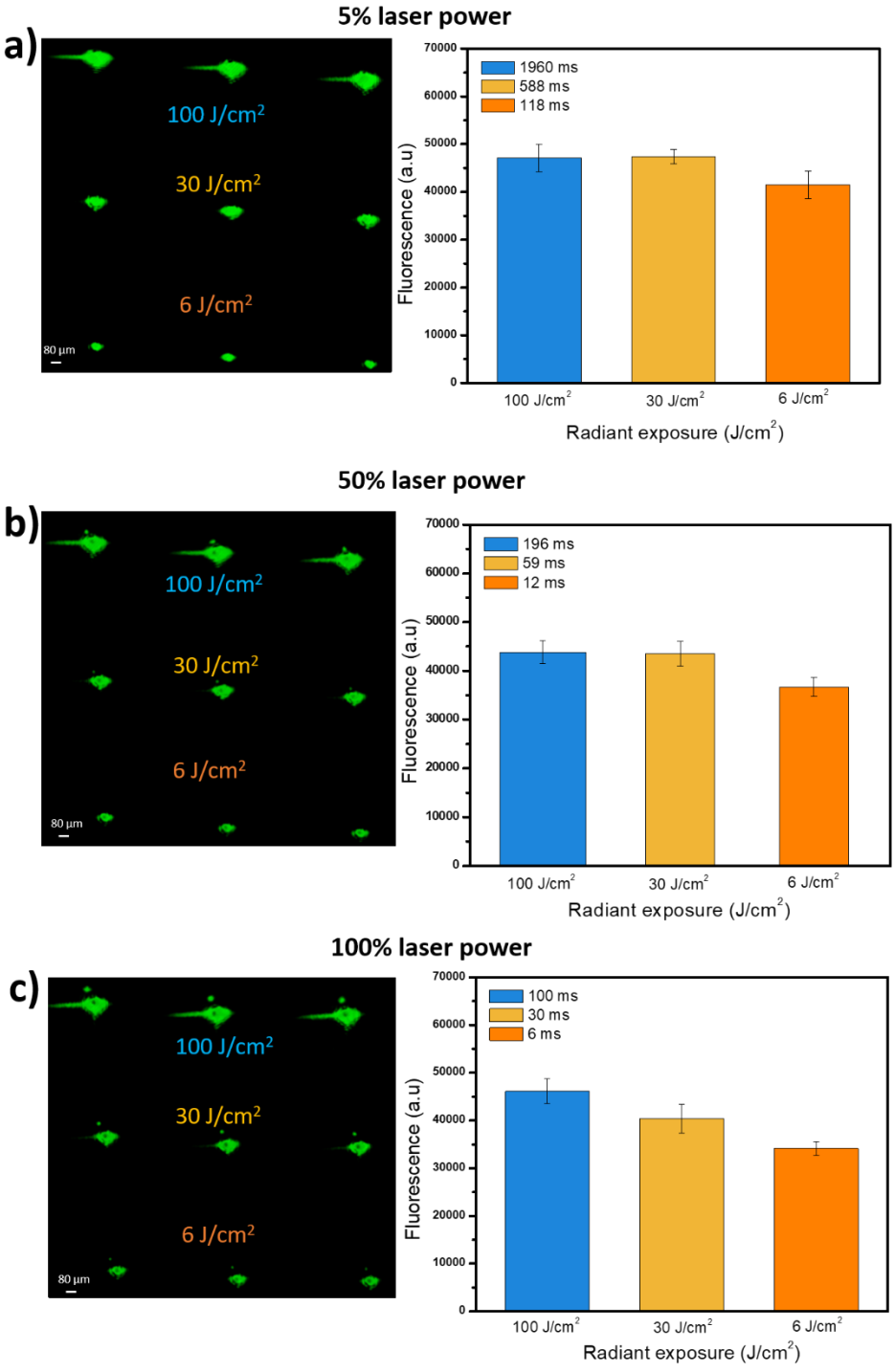


Figure 9 - Comparative study of exposure conditions used to synthesize a 25-mer sequence using the laser system. Different laser powers—(a) 5% (~51 W/cm²), (b) 50% (~511 W/cm²), and (c) 100% (~1022) W/cm²)—were used to synthesize the sequence using radiant exposures of 6, 30, and 100 J/cm² with different exposure times.

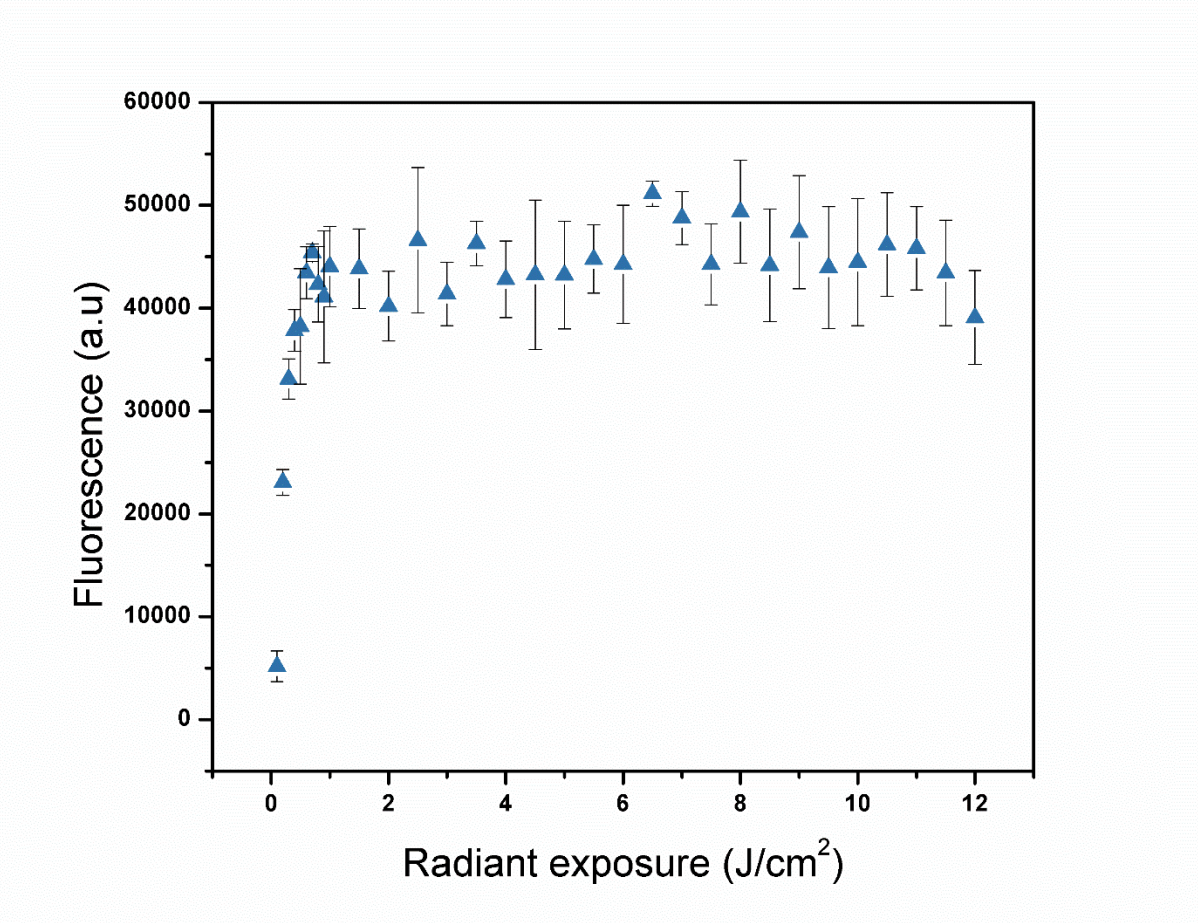


Figure 10 - Hybridization intensities for 25-mer sequence arrays were synthesized with an exposure gradient. The synthesis was done using 5% and 1% laser powers, with exposure times ranging from 10 to 235 ms. The signal intensities correspond to the average of five features.

The photochemistry of NPPOC derivative removal involves two stages. First, UV light induces triplet sensitization of the nitrophenyl core, forming an aci-nitro structure. Then, a base, in our case imidazole, abstracts a proton, triggering a β-elimination process that cleaves the carbonate bond linking the NPPOC group to the 5'-OH.[5] Therefore, under fast deprotection conditions, such as those used in the laser system, the rate of proton abstraction was expected to be a limiting factor. As observed in Figure 3, more homogeneous features with higher signal intensities are obtained using longer reaction times and low powers. This is particularly significant for features synthesized with 6 J/cm², which exhibit signal intensities approximately 18% higher for a reaction time of 118 ms compared to 6 ms. Another phenomenon that could contribute to this observation is the jitter of the beam. During longer exposures, there is more time for the beam to move around the central position, which can smooth out any sharp artifacts, such as those arising from interference phenomena. It's important to emphasize, however, that even with such ultra-fast reaction times, the synthesis yields

obtained are comparable to those achieved in the current system used at UniVie. This system employs LED devices as light sources (irradiance at 365 nm: 100 mW/cm^2), with reaction times at least 80 times longer.

4. Conclusions and outlooks

In summary, we have successfully developed a prototype laser device for DNA photolithographic synthesis, operating with a 405 nm laser and tested on glass substrates. The synthesis yields achieved with this laser exposure device were comparable to those obtained using the 365 nm LED-based device currently used at UniVie. However, due to the significantly higher intensities achieved with the laser, the necessary reaction times were significantly reduced. Notably, we demonstrated that good yields can be obtained even with exposure times lower than 10 ms, suggesting compatibility with wafer-scale synthesis and enabling the illumination of thousands of spots in minutes. For example, considering the stabilization time between spots, illumination of 1000 positions could be achieved in less than 3 minutes.

Despite demonstrating the possibility of laser-assisted DNA synthesis at 405 nm, several issues still require attention. Some fluorescence signal artifacts were observed, and there is high variability in spot size within a wide range. Consequently, we cannot yet accurately determine the exact radiant exposure required for photodeprotection of SPh-NPPOC at 405 nm. We anticipate that these artifacts could be addressed by using lasers with better optical properties. In the meantime, experiments will be conducted using an LED at 405 nm to obtain an accurate exposure gradient for SPh-NPPOC.

Moving forward, optimization of synthesis conditions is underway at UniVie using the current prototype. This optimization includes exploring conditions such as the concentrations of the base in the exposure solvent, which could improve synthesis homogeneity at short reaction times. Additionally, experiments are being conducted to synthesize multiple sequences in parallel, further demonstrating the efficiency and throughput of the laser device.

Having demonstrated the suitability of the laser approach, the specifications, costs, and requirements for the development of the wafer-scale laser exposure system are currently being investigated at VTT.

5. References

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