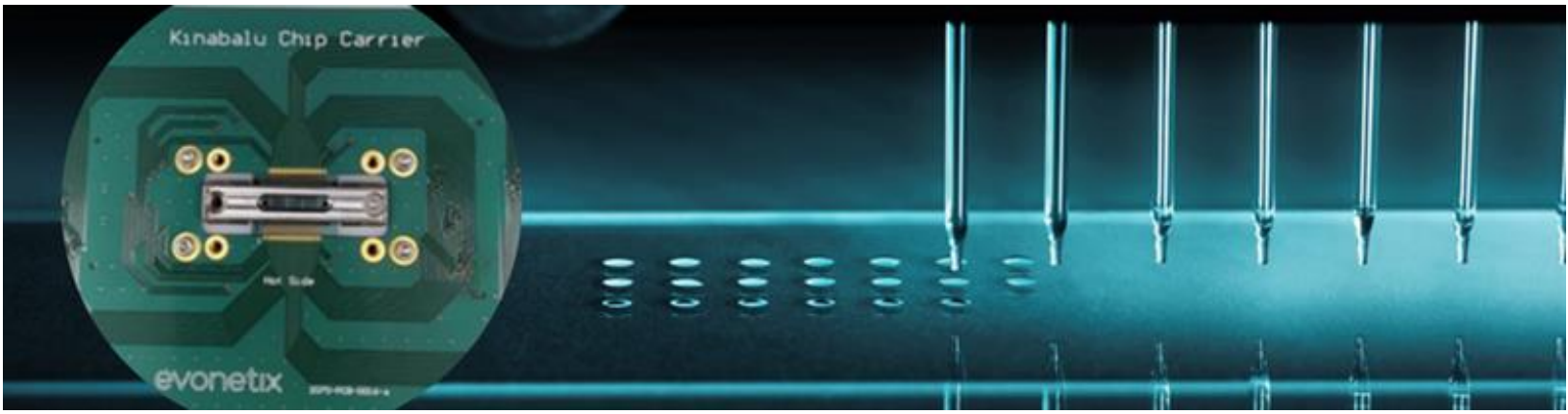
Application Note



Surface functionalization of EVONETIX's semiconductor chips for DNA synthesis using SCIENION's precision dispensing technology

Dan Bratton[1], Evangelia-Nefeli Athanasopoulou[1], Mat Jennison[1], Nicoló Ferri[1], Timothy King[1], Kristina Mykhailiuk[2], Stephani Dulac[2], Svenja Plesshoff[2], Wilfried Weigel[2], Julian Kirby[3]

[1]Evonetix Ltd, Cambridge, UK
[2]Scienion GmbH, Berlin, GER
[3]Scienion UK Ltd, Portsmouth, UK

Abstract

In this study, Evonetix Ltd and Scienion GmbH worked together on a scalable process for highly parallel DNA synthesis on silicon semiconductor chips. Using Scienon's precision dispensing technology, a linker to initiate DNA synthesis on 100 µm diameter gold sites has been printed on Evonetix's DNA synthesis platform. As a result of the study, the linker could be accurately placed on the reaction sites, thereby minimizing overspill and minimizing crosstalk between adjacent DNA sequences. Evonetix successfully demonstrated the functionality of the coated chips during DNA synthesis studies.

Introduction

Evonetix Ltd is a Cambridge-based company developing novel semiconductor technology to generate high fidelity DNA at scale. The desktop DNA synthesis platform provides the ability to

synthesize DNA at unprecedented accuracy, scale, and speed. DNA synthesis takes place on independently temperature-controlled reaction sites realized on a semiconductor chip (Figure 1). A thin gold layer is deposited onto the reaction sites which in the next step will be functionalized with an in house developed base coating. The coating enables covalent attachment to the gold sites and a later immobilization of a linker. The linker carries a reactive head group for binding of the first phosphoramidite to start the ssDNA synthesis. The chip itself is assembled into a printed circuit board. A fluidic chamber is placed on top of the chip.

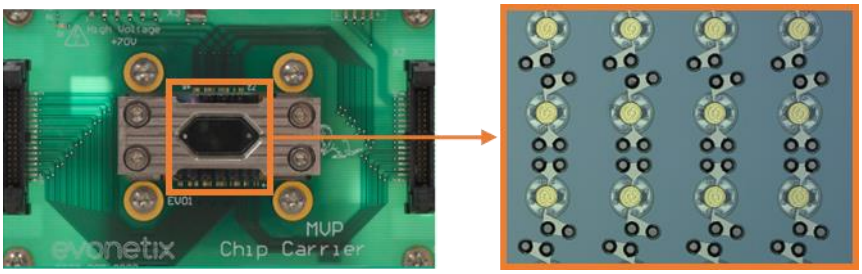


Figure 1: Printed circuit board (left) carrying an assembled semiconductor chip with independently temperature-controlled reaction sites (right).

Scienion GmbH is a complete solution provider for the development and manufacturing of miniaturized multiplex assays for diagnostics and other fields of life sciences. Scienion's core technology is based on non-contact reagents' deposition in the picoliter and nanoliter range, starting from 10 pL/drop. Besides instrumentation and consumables, Scienion offers a range of custom development and manufacturing services. In this application note, a Proof of Principle (POP) study was performed to understand the capabilities of the dispensing process on Evonetix's semiconductor chips. Finally, Scienion's dispensing technology was successfully applied for the functionalization of Evonetix's DNA synthesis platform. Thereby, a linker was dispensed onto 100 µm big pre-coated gold reaction site to initiate DNA synthesis on the semiconductor chip.

Figure 2: (Left) The sciFLEXARRAYER SX precision dispenser with integrated sciDROP PICO technology used in this study. (Right) Enlarged image of the dispensing unit of the sciFLEXARRAYER SX, including (1) Piezo Dispense Capillaries, (2) the head camera used for imaging targets after printing and (3) the drop high resolution camera allowing to visualize and quantify drop parameters.

Results and discussion

Preliminary study

First, Scienion's custom development services were applied, and a preliminary study was conducted to understand the interplay of the chip surface, spotting volume and spot diameters.

Influence of the surface wettability on the spot diameter

To estimate the drop volume needed to cover 100 µm diameter gold reaction sites of Evonetix's chip, water droplets of different volumes (80 – 320 pL) were dispensed onto different targets using Scienion's precision dispensing technology. Pictures of deposited droplets (spots) were taken by the head camera and their diameter was analyzed by the sciFLEX software. Additionally, contact angle measurements with water were performed on the support material of interest to analyze their wettability properties. Finally, the impact of wettability properties on the spot diameter was evaluated.

As main supports to investigate, *uncoated Au slides* (CA: 96°) and *base coated Au slides* (CA: 95°) were used to simulate the gold sites. Both slides were provided by Evonetix. For comparison, *sciCHIP H2* (CA: 106°) and *sciCHIP Epoxy* (CA: 53°) slides were used as additional reference surfaces.

The interplay of droplet volume and spot diameter on surfaces with different wettability properties is shown in Figure 3. Higher hydrophilicity of the sciCHIP Epoxy surfaces results in significantly larger spot diameters. Similar spot diameters are obtained for *sciCHIP H2* and *Au blank slides* due to comparable wettability properties. Slightly larger diameters (7-17%) have been measured for *coated Au slides* despite similar values for contact angle. About 210 pL big water droplets must be dispensed onto *coated Au slides* to reach the spot diameter of 100 µm, while about 320 pL big water droplets are needed to reach the same spot diameter on *uncoated Au slides*. These results were used in the next study to evaluate if similar droplet volume is needed when dispensing different buffers.

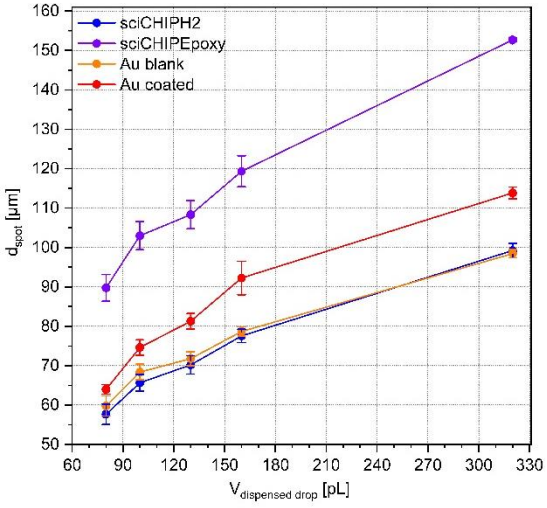


Figure 3: Spot diameters (y-axis) in dependence of dispense volumes (x-axis) for sciCHIP H2, sciCHIP Epoxy slides Au blank, and base coated Au chip.

Influence of different buffer condition on spot formation (spot size)

The 100 µm diameter reaction sites on Evonetix's semiconductor chips needed to be covered with a DNA linker. It was investigated which of three available buffers is the most suitable for the immobilization of the DNA linker onto pre-coated gold sites. Furthermore, the drop volume was varied between 200 pL and 3200 pL to evaluate the optimal dispensing volume. The tests were conducted on coated Au slides to investigate the impact of buffer composition and drop volume on spot formation.

200 – 3200 pL/spot were dispensed as a sequence of 200 pL/drop/round with 2 min waiting time in between the rounds. The spot diameter was measured after each sequence (Figure 4). *Evx buffer* (provided by Evonetix) and *sciSPOT B2* (provided by Scienion) were used as buffer reagents. Additionally, *Evx buffer* was mixed with *sciSPOT B2* to combine the properties of both buffers.

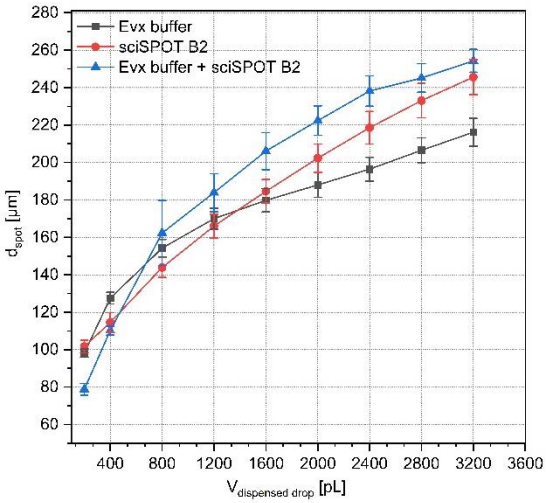


Figure 4: Spot diameters obtained after sequential dispensing (200 pL/round) of Evx buffer, sciSPOT B2 buffer and their mix onto coated Au slides.

With *Evx buffer*, a spot diameter of 98 ± 2 µm was reached when dispensing 200 pL, and 127 ± 3 µm when dispensing 400 pL. After deposition of 800 pL, the spot diameter increased by less than 10% after each additional round. With *sciSPOT B2 buffer*, diameters of 102 ± 2 µm were reached for 200 pL, and 115 ± 5 µm for 400 pL. The spot diameter increases almost linearly by 5-13% with each additional dispensing round. With *Evx buffer + sciSPOT B2 buffer*, a diameter of 79 ± 3 µm was achieved when dispensing 200 pL and 110 ± 2 µm for 400 pL. The spot diameter increases almost linearly between 200 – 800 pL (40-47% increase) and 800 – 3200 pL (4-13% increase).

The volume needed to cover 100 µm of coated gold surface is about 200 pL. All three buffers tested could cover the aimed spot diameter. It was decided to use the combined properties of *Evx buffer + sciSPOT B2 buffer* for deposition of DNA linkers to achieve optimal immobilization.

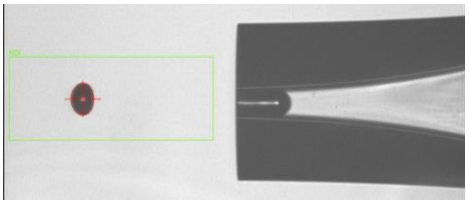
Functionalization of Evonetix's chip using Scienion's precision dispensing technology

Evonetix's chips with independently temperature-controlled reaction sites were functionalized with the base coating at Scienion. The chips were assembled into chip carriers, connected to a fluidic fixture, cleaned, and functionalized with a reactive and thermally stable coating according to a protocol provided by Evonetix. In the next steps, the carriers with the chips were placed onto the target deck of a sciFLEXARRAYER SX (Figure 5). The target deck was cooled to 8°C and the relative humidity was set-up for 65%.

Figure 5: Four Kinabalu chip carriers with coated chips placed on the target deck of the sciFLEXARRAYER SX.

As reagents, ssDNA-Cy5 and a linker were printed onto the reaction sites. The first reagent is used as positive control, while the second reagent allows immobilization of the first DNA-base during the later synthesis process. About 200 pL of the reagents are required to cover the 100 µm diameter reaction sites without overspill to the neighboring area (Figure 6), verifying the results of the preliminary study with different buffer conditions.

(a) 201 pL/drop of linker in phosphate buffer



(b) 204 pL/drop of ssDNA-Cy5 in *Evx buffer* + *sciSPOT B2* mix

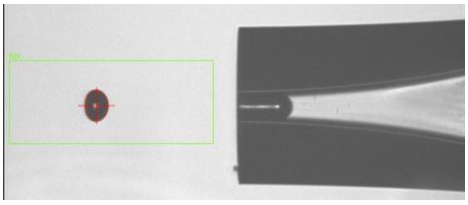


Figure 6: Images of 200 pL drops from the drop camera of the sciFLEXARRAYER SX.

The reaction sites on the chip are arranged in three fields with 3x3 sites per field. The pitch between the sites within a field is 400 µm in both directions and the gap between the fields is 2800 µm. During the dispensing process, position 3/3 of each field was left empty, ssDNA-Cy5 was deposited into the position 2/2 (middle spot), and linker was deposited onto the remaining sites (Figure 7).

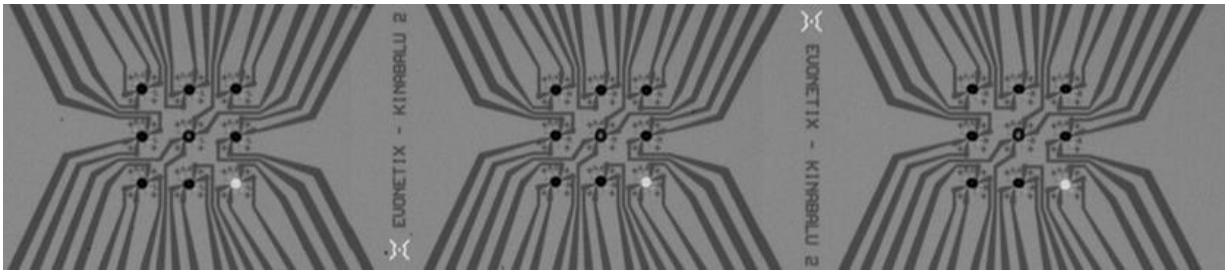
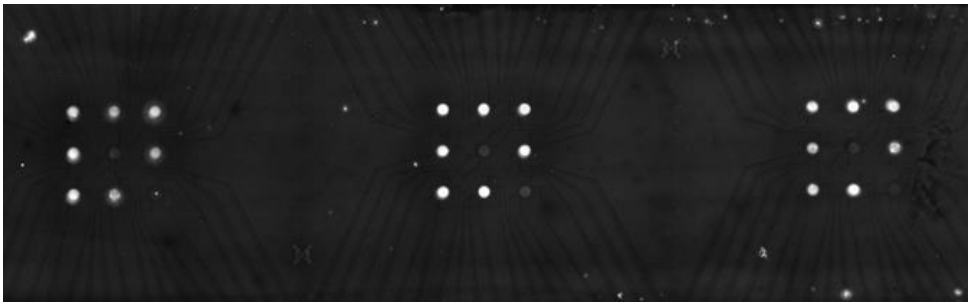


Figure 7: Array layout: Images of empty site (position 3/3), positive control ssDNA (position 2/2) and linker (remaining positions) deposited onto coated gold reaction sites.

Reagents were deposited in a sequence of two rounds: 200 pL of the reagent, and after 5 min another 200 pL of the same reagent were deposited per site to achieve a higher accumulation of the reagent. The resulting average spot diameter was 120 µm ± 10 µm which indicate a minimal overspill. After the deposition, chips were incubated at 70% rel. humidity at room temperature overnight. The reagent surplus was removed from the surface by a wash procedure.

ssDNA was synthesized on all gold sites functionalized with the linker (except position 2/2 with the positive control as well as empty position 3/3) and hybridized with ROX-labelled complimentary ssDNA. Fluorescence images of ROX-labelled ssDNA using a Leica DMi8 Fluorescence microscope demonstrate that the synthesis of targeted ssDNA on previously linker functionalized reaction site was successful (Figure 8a). The measured intensity of 5715 ± 395 RFU is 5 times higher compared to the unfunctionalized reaction site at position 3/3 (1130 ± 90 RFU). The fluorescence intensity of the positive control ssDNA-Cy5 at position 2/2 is 9 times higher compared to the uncoated reaction site (positive site: 17117 ± 4248 RFU; unspotted site control: 1911 ± 87 RFU).

(a)



(b)

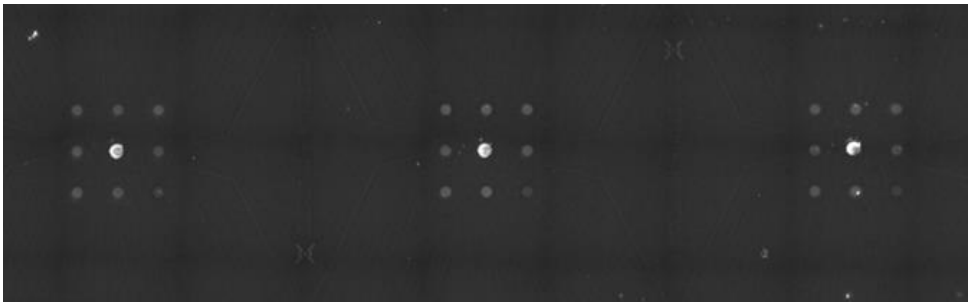


Figure 8: Fluorescence images taken of the same chip using a Leica DMi8 Fluorescence microscope (a) after hybridization of ROX-labelled complimentary ssDNA with ssDNA synthesized on linker functionalized sites (LED excitation 560 nm, emission filter 592-668 nm 5x magnification lens); b) fluorescence image of positive control (LED excitation 635 nm, emission filter 666-724 nm 5x magnification lens).

In a subsequent study 3 volumes of linker were deposited on the gold sites to investigate how the density of immobilized linker may impact the amount of ssDNA synthesized: 2 drops, 6 drops and 8 drops (dispensed as 200 pl/drop). After the hybridization with the ROX-labelled complementary ssDNA, fluorescence images were taken (Leica DMi8 Fluorescence microscope) and fluorescence intensities measured (Figure 9). It can be concluded that with the increasing amount of linker immobilized, the fluorescence intensity and thus the amount of synthesized ssDNA increases. Deposition of 6 drops/spot results in a fluorescence intensity which is:

- Almost 3x higher compared to fluorescence intensity achieved after deposition of 2 drops of linker
- In the same order of magnitude compared to fluorescence intensity achieved after deposition of 8 drop/spot of linker.

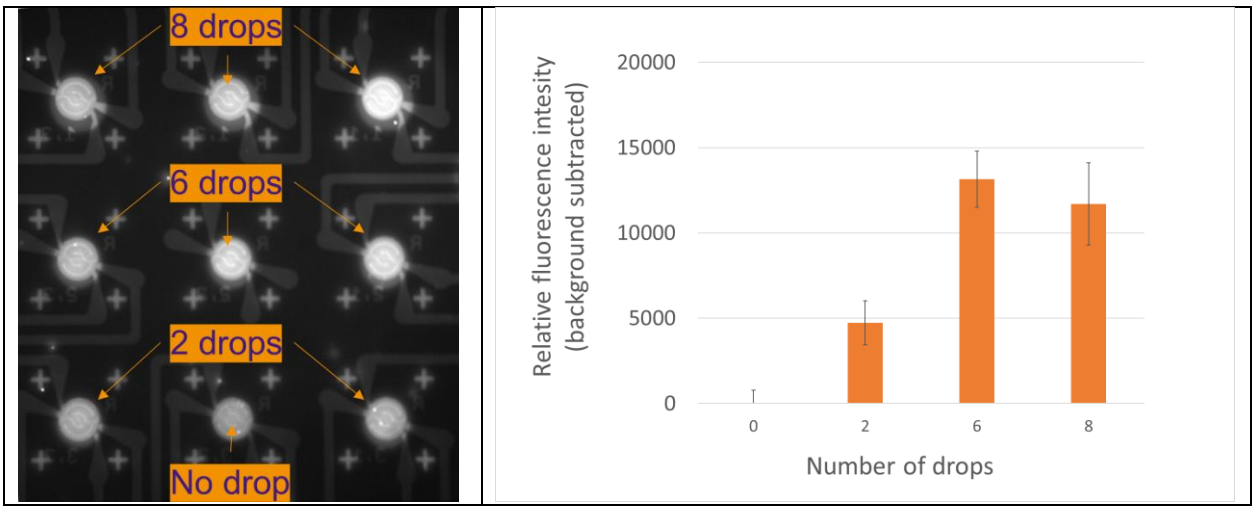


Figure 9: Fluorescence image after hybridization of ROX-labelled complimentary ssDNA with ssDNA synthesized on linker functionalized sites (left). Fluorescence intensity was measured to evaluate the impact of different amount of immobilized linker (LED excitation 560 nm, emission filter 592-668 nm 10x magnification lens).

Conclusion

Scienion's precision dispensing technology was successfully utilized for the functionalization of 100 µm big gold sites of a DNA synthesis platform developed by Evonetix. Different spotting parameters, including buffer and linker concentration, were evaluated to determine the best parameters for the DNA synthesis. We were able to demonstrate that printing of 2-3 drops (200 pL) of a linker on the same reaction site resulted in an increase in signal. Printing of six drops is sufficient to saturate the reactive site in the printed area, beyond which there is no further increase in signal.

Contact Us

Scienion GmbH
Wagner-Régeny-Str. 15
12489 Berlin
Tel: +49(0)30-6392-1700
support@scienion.com
www.scienion.com

Registered Office: Berlin
AG Berlin (Charlottenburg), HBR 259454
Managing Director: Dr. Frauke Hein
VAT ID No.: DE813228678
WEEE-Reg.-Nr.: DE85315076

Commerzbank AG
Bank Code: 100 400 00
Account No.: 5272380
IBAN: DE69 1004 0000 0527 2380 00
SWIFT/BIC Code: COBADEFFXXX