Lab on Paper: Adapting Quantitative Chemical Techniques for Use in Low Resource Areas

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Test Spotting Report

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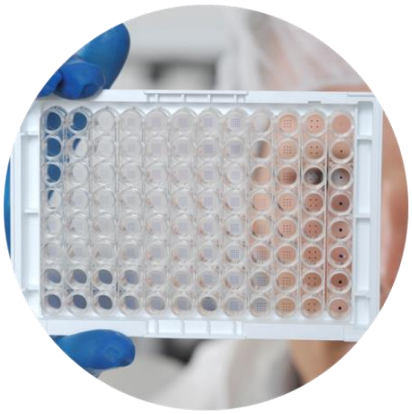

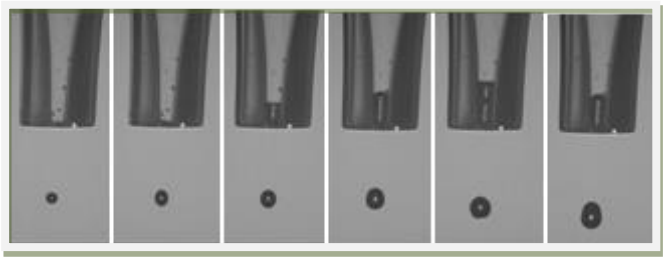

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ABOUT SCIENION

Scienion was founded in 2001 in Berlin, Germany as a spin-off company of the Max Planck Institute for Molecular Genetics. We provide our customers with **high quality dispensing (ultra-low volume) and micro arraying solutions** and **contract manufacturing service** but also give extensive **application support** to help you shift your ideas from R&D to production.

- Scienion is one of the key players in non-contact dispensing in the **nano- to picoliter range.**
- Scienion offers an integrated portfolio comprising **hardware, software, consumables** as well as **development** and **contract manufacturing services**.
- Scienion's team of application specialists has extensive know-how in application support in **spotting** and **micro arraying** technologies.

INTRODUCTION

The sciFLEXARRAYER is a **non–contact** dispensing system, which allows spotting and dispense of liquids in the **pico- to nanoliter range.** Two different technologies can be implemented for this purpose:
1- sciDROP PICO: A piezo-driven dispenser made off high grade inert glass with round orifices. This prevents liquids with high salt concentrations from crystallizing as happens in nozzles with corners (i.e. silica-etched nozzles). Various nozzle sizes ensure volumes from 50 – 800 pL. In addition, four standard coatings are available to ensure a stable dispense of all kind of biological samples and chemicals.
2- sciDROP NANO: A solenoid valve controlled dispenser with volumes that range from 20 – 200nL per drop.
Unlike other systems available on the market, Scienion's dispensers do NOT increase the sample temperature, nor are any additives required!
Both bulk dispensing and aspirate / dispense modes are available on our systems.

WHY CHOOSE SCIENION

- One core technology in all systems (S1 through S100)
- High reproducibility and superior spot morphology
- No contact with spot surface allows for printing onto sensitive surfaces
- Optical measurement of drop size thus, full control drop volume
- Ability to print onto 3D structures and supports with complex geometries (e.g. biosensors)
- Precise dispensing in picoliter to nanoliter range
- No need for printing additives
- No cross contamination
- No software scripting or compilation. Easy to use GUI
- Easily scalable from R&D to high-throughput production
- Life video streaming of the production process (live view cam)

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VARIOUS OPTIONS

Ceramic vacuum holder

Fixes the target patches firmly during spotting. Can be used on slides, sheets (NC, Nylon, etc.), MTP, sensors, etc.

Find Target Reference Points (FTRP) software

This function spots an array in reference to a start point (X). It detects one or more fiducials of any shape or size (+; Δ) having sufficient contrast. A single fiducial must fit inside a single field of view. The implementation of two or more fiducials, preferably as far apart as possible, will enhance the spotting precision due to correction of target rotation. The markers can be located in a single field of view or in several camera positions allowing for angle correction. This positioning defines a reference point to which the array is related to.

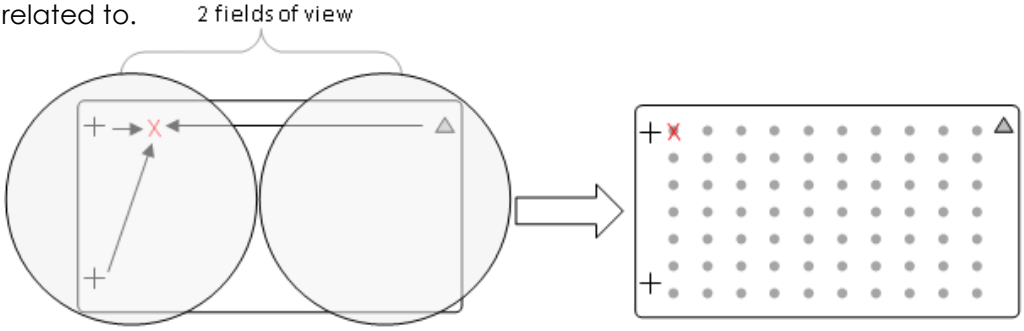


Figure 1: *Find Target Reference Points*

Scan Spot Area & QC software

Moves the camera over the spot area and makes a picture either of the entire target or only the spotted fields. The tool is also capable of stitching together several pictures to one big image. Image can be further analyzed with Analyze Spot Area or with third party software.

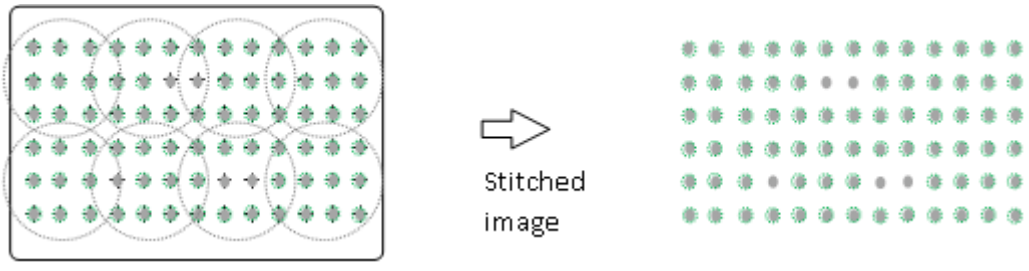
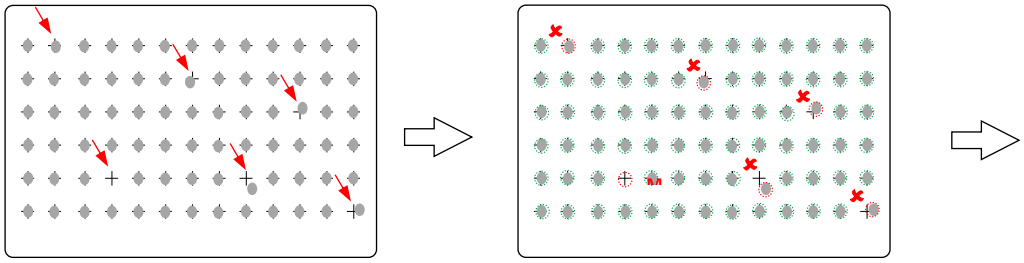


Figure 2: *Scan Spot Area*

Online Array QC Software:

Imaging and Image analysis software. Image(s) of the target area will be automatically analyzed for roundness of spots, missing spots and positional accuracy of spots. Inter and intra array spot analysis. All data will be given Graphical and numerical reports.



Report

1	Position accuracy
2	Spot area
3	Roundness
4	Global shift of array
5	Search for dried shapes in a spot (i.e. crystals)

Graph showing deviation in spot positions

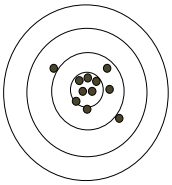


Figure 3: *Online QC Software*

Drop Control:

This tool provides automatic recognition of the dispensed drop and enables the control of the dispense performance during operation. It further regulates the speed (position) of the drop, thereby stabilizing the printing process.

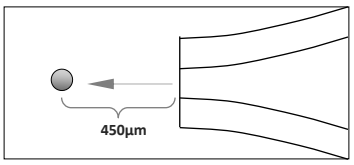


Figure 4: Drop Control

Upgrade to Volume Control:

In addition to automatic recognition of the dispensed drop, this tool measures and controls the volume of the drop during operation. It regulates both the speed and volume of the droplets, ensuring maximum stability of the dispense process and comparability between different runs.

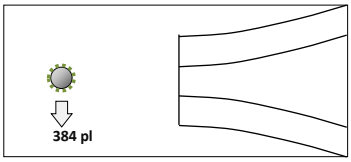


Figure 5: Drop Volume

AIMS & OBJECTIVES

In this pilot study we aim to dispense the client supplied solution precisely to localized areas on client supplied membrane. The intention of this initial test spotting is to demonstrate the capability of Scienion´s sciFLEX technology to deliver droplets in the pico-liter scale to accurately fulfill the range of nano-liter loading requreiments of this project.

Scienion evaluated suitable camera based spotting coordinate detection and developed a printing routine to precisely deposit all sample volumes.

MATERIALS PROVIDED

- Thiosulfate solution
- Several sheets of printed membrane
    - Sheets were bent in shipment - attempts were made to flatten them and all dispensing locations were taught ~1 cm from target holder surface (standard height is 450 um from substrate surface) to avoid contacting the substrate.

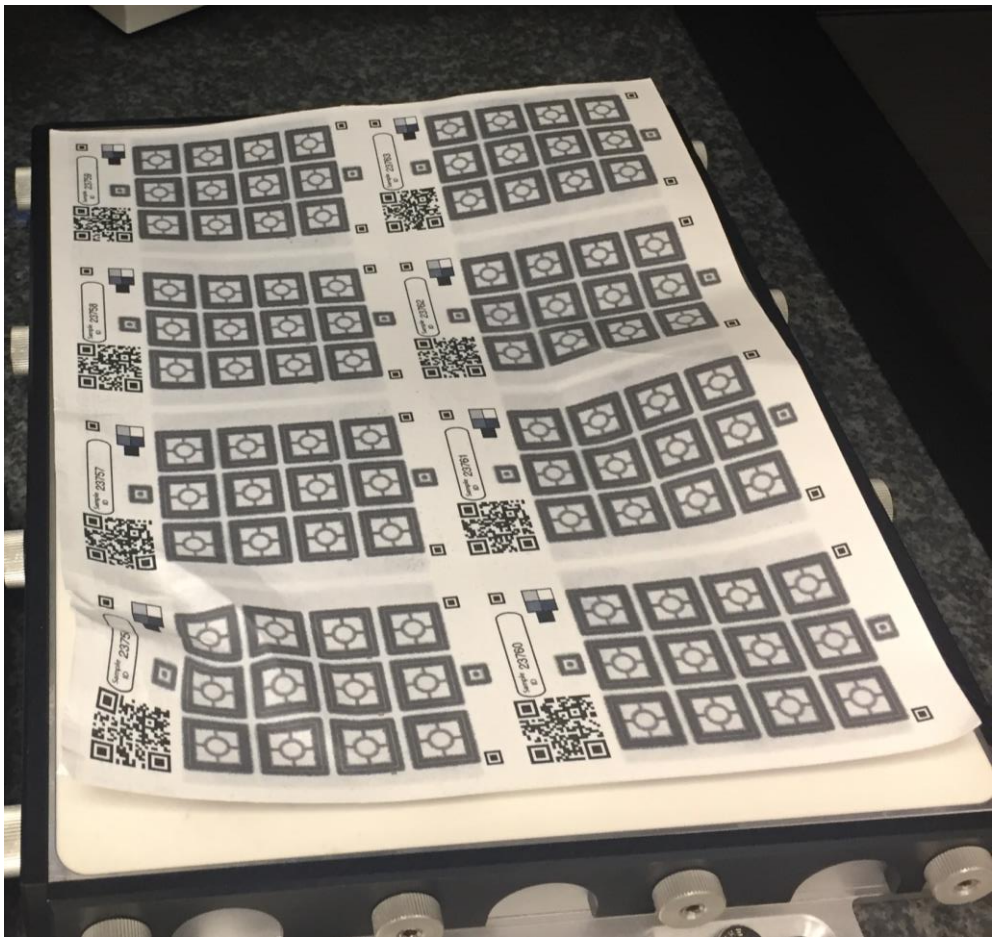


Figure 6: Example of *sheet damage incurred in shipping.*

SPOTTING CONDITIONS

- Humidity: 50%
- Ambient temperature: 22 C
- Source plate temperature: Ambient
- Dew Point or manual cooling: None

MACHINE AND PD

- sciFLEXARRAYER S3
- PDC 70 Type 4(ID 23811)
    - Voltage = 95
    - Pulse Width = 48

Figure 7: *sciFLEXARRAYER S3: Non-contact microarray spotter with spindle drive step motor axis system*

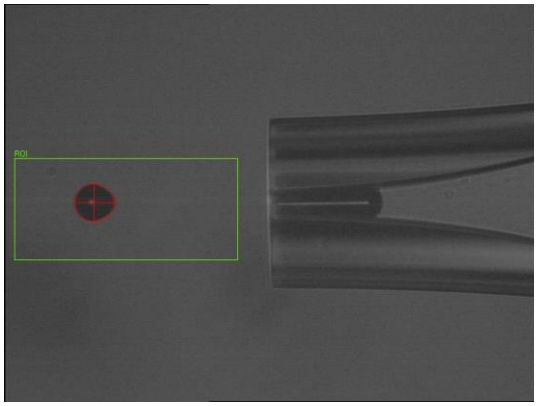


Figure 8: *Demo PDC dispensing 296 pL droplets of purified water at 500 Hz*

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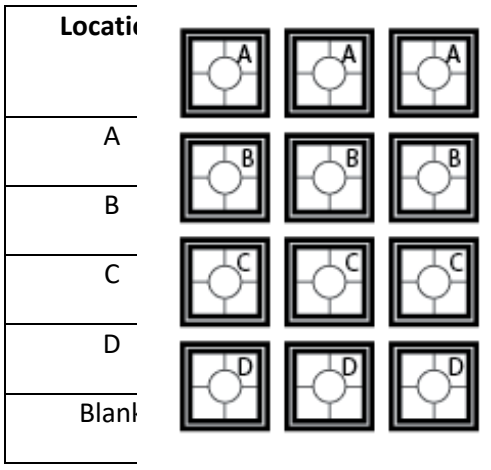
ARRAY LAYOUT

- 2 x 4 Spot areas with a single field of 4x3 spotting locations
- Dot pitch between spots: 18000µm (X:Y)
- Multiple drops per spot defined by volume entry software function
    - This function calculates the number of droplets required to be within ½ single drop volume of target spot volume



ense volumes.

Figure 10: Diagram of dispense locations

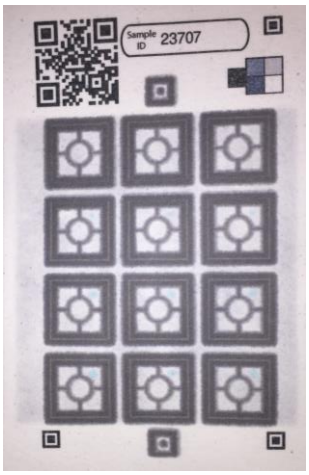


Figure 11: *Image of target substrate spot area*

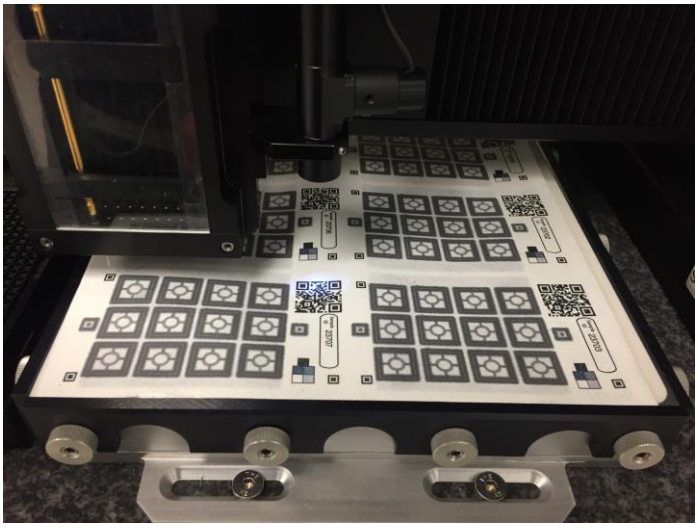


Figure 12: Sheet shown on target holder in print orientation.

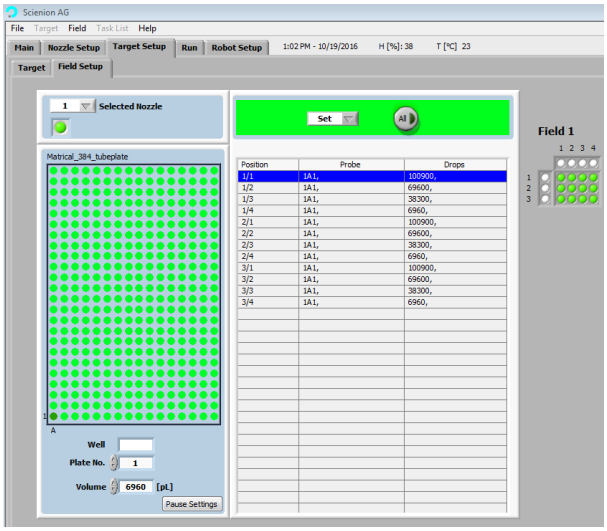


Figure 13: Screenshot of field setup.

RESULTS – PRINTING

FTRP setup

To accurately deposit sample spatially two fiducials were chosen with appropriate contrast and size in separate areas on the sheet to increase tolerance for human error in sheet placement.

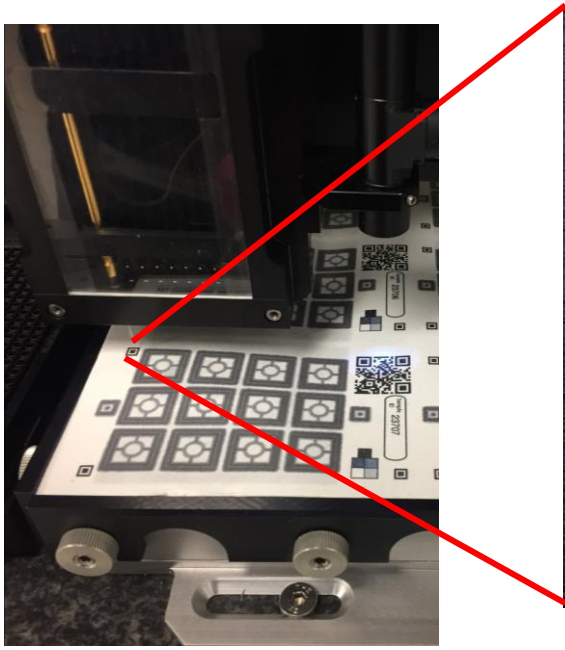


Figure 14: Nozzle shown positioned at fiducial #1.

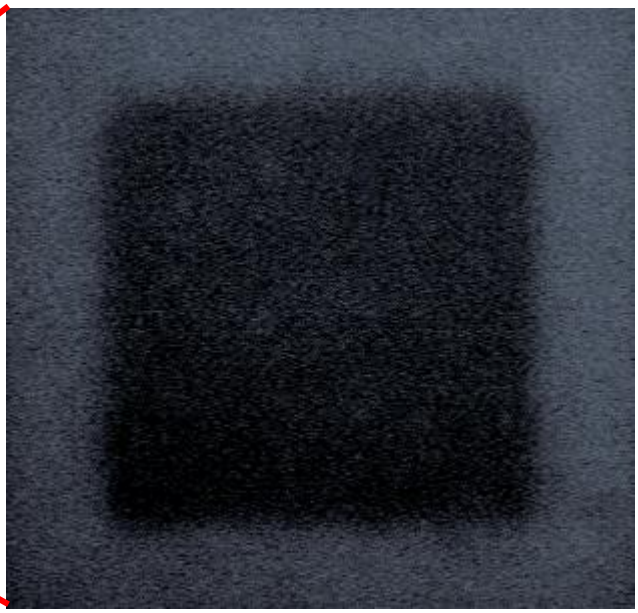


Figure 15: Fiducial 1 as imaged by head Camera.

Initial spot positioning and accuracy test

One sheet was printed using this **FTRP** setup. Initial dispense was on target but the as the printing progressed dispense was observed to stray and bead-up onto waxy surface. Upon further inspection, wax pattern and printed ink patter (used for fiducial recognition) were not aligned to eachother. For all subsequent testing, a brief manual alignment was done on all sheets.

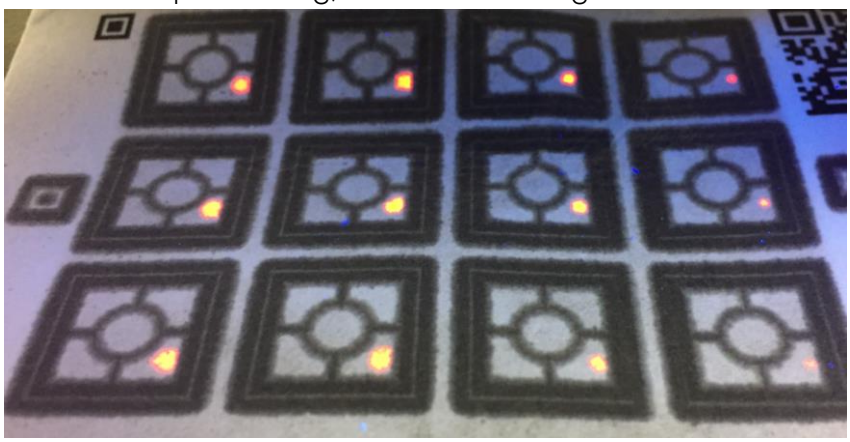


Figure 16: Due to colorless and quick drying sample characteristics, dispense of fluorescent beads was tested for dispense location verification.

PDC performance

Drop performance was not noticeably different for thiosulfate sample than for water. No adjustment of drop parameters was required to dispense the sample. Drops were consistently stable with sub 1.0% CV and no film formation was observed during testing.

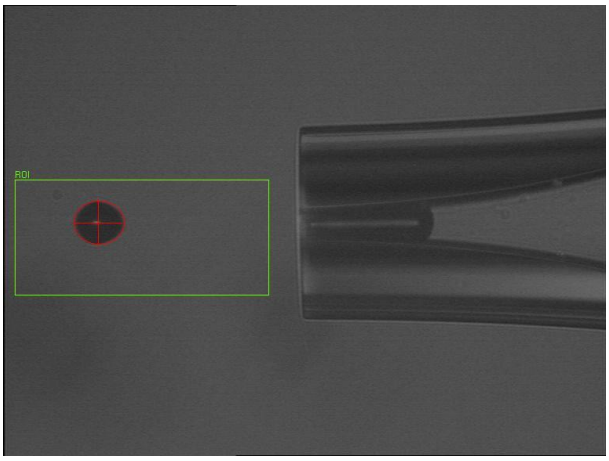


Figure 17: Drop Camera image of sample dispense.

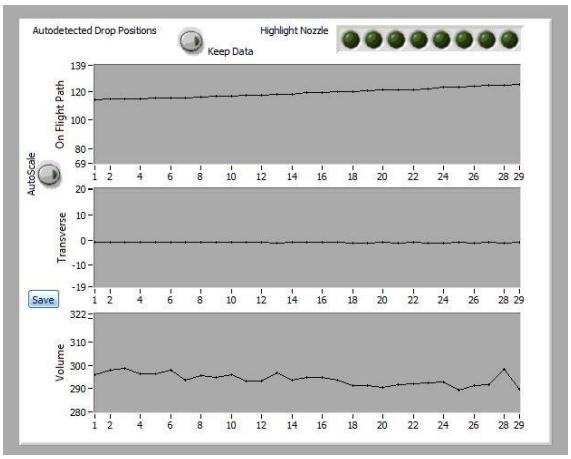


Figure 18: Dropgraph of sample dispense.

Data gathered by **Drop Volume** function originates from analysis of dropcam images. Due to the optical nature of these measurements, they can be affected by changes in ambient lighting and care should be taken to minimize this. A slight decrease in drop volume and speed (On Flight Path in figure 18.) was observed over the course of runs. This is due to sample conditioning of the nozzle and is reversible with standard cleaning procedures.

An **Autodrop Detection** was implemented into the run task list to allow for recalculation of the number of drops to be dispensed if drop volume is significantly changed.

CONCLUSION

Feasibility study of spotting supplied sample onto supplied sheets could be performed successfully. Spotter equipment sciFLEXARRAYER S3 and dispense technology sciPICO could accomplish printing of desired 4x3 array to each card in approximately 15 seconds with a single PDC 70 operating at 500 Hz.

RECOMMENDED MACHINE CONFIGURATION

Scienion recommends a sciFLEXARRAYER S3 with sciDROP PICO dispense technology.
In addition, the system should be equipped with the following options:

- sciDROP PICO (number of channels to be decided)
- Nozzle size: PDC90, Coating Type 4
  - Further nozzle size and coating optimization may increase stability of drop size and decrease total dispense time in production
- Drop control with upgrade to drop volume
- Head Camera
- Ring light for diffused illumination to increase contrast of wax patterning with Head camera
- Humidity control to control substrate wetting and evaporation rate
- Cooling device for source plate (target also coolable)
- Dew point control to avoid sample evaporation in source plate

Best regards,
Your Scienion Team

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