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Nano-Plotter Application Notes

- Non-contact picolitre dispensing for high-quality spotting
- Selection of pipette types to match the sample, not vice versa
- Up to 16 independently operating piezoelectric dispensers
- Many extras such as chilling, humidification, alternative dispensers, automatic target recognition and more

The GeSiM Nano-Plotter – Designed with special applications in mind

The micropipetting instrument GeSiM Nano-Plotter with its unique piezoelectric pipettes (that deliver droplet volumes ranging from 50 to 400 pl) and its various accessories is of course primarily used for the printing of microarrays, the functionalisation of biochips and for multi-parameter assays. Samples typically contain DNA, proteins or other biomolecules.

In addition to these “core applications”, the flexibility of soft- and hardware makes sure that the Nano-Plotter has become a valuable and reliable companion in fields as diverse as drug discovery, microfluidics, and printed electronics. Because of this, It has made its way around the world for more than twenty years.

Get an overview from the Nano-Plotter brochure or visit www.gesim.de.

Be inspired by the versatility of the Nano-Plotter and harness our sound knowledge!

Here is an overview over the current GeSiM Nano-Plotter application notes:

Microtitre plate cooling

Adapter plates for more efficient microtitre plate cooling

Metal plates exist into which accurately fit to different microtitre plates, to optimise thermal coupling between the cooling chuck and the microtiter plate and to minimise temperature differences between wells.

Accuracy of piezoelectric GeSiM pipettes

Gravimetric determination of the accuracy of GeSiM piezoelectric pipettes

Here we show how GeSiM's piezoelectric dispensers work in terms of volume accuracy and variation.

Compound dilution and CVs of GeSiM piezoelectric pipettes

This method is also called direct titration, as opposed to serial dilution, as you fill an amount of buffer or solvent into wells, and the Nano-Plotter dispenses different volumes of stock solution into these wells, thus obtaining all different dilutions of the compound. The method has also been used to determine the accuracy of the GeSiM piezo dispensers using fluorescence.

Printing of alternative geometries

Line printing for the manufacturing of lateral flow assays

The GeSiM Nano-Plotter has a built-in line printing function: the head accelerates to the start position and then spots at constant speed and droplet frequency before it decelerates. This is a prerequisite for the manufacturing of the popular lateral flow assays.

Bitmap Converter – Print images with the Nano-Plotter

The Bitmap Converter is an application that converts bitmap files (.bmp, .jpg, .png,.tiff etc.) so that they can be printed on a Nano-Plotter using the programme system SpotFrontEnd/ SpotFromFrontEnd (SFE/SFFE), effectively turning it into an artist.

Printed electronics

Ink-jet printing of commercial conductive inks using GeSiM piezo tips

Printed electronics is a hot topic in microelectronics, where electrodes can serve e.g. as actuators and sensors on foils, but also in biology where electronic actuators can be incorporated into 3D-printed bioinks. (The larger GeSiM Multi-Material-Printer ("MMP") extends the capabilities of the Nano-Plotter by adding many more tools, some of which enable the extrusion of highly viscous polymers, e.g. for polymer electronics. Please see the brochure or ask.)

Microfluidics and 3D structures

Piezoelectric coating of micro-needle arrays using the GeSiM Nano-Plotter

Whereas the GeSiM microcontact printers can easily create micro-needle arrays via nanoimprint lithography (see separate brochure), the Nano-Plotter is the perfect tool to functionalise micro-needles with biochemical molecules

Ink-jet printing of PDMS solutions using GeSiM piezo tips

Ink-jet printing of polyvinyl alcohol (PVA) solutions using GeSiM piezo tips

The GeSiM ink-jet printing technology can be used for additive manufacturing, as it allows the deposition of small amounts of material at specified positions anywhere on a substrate. Printing multi-layer structures then result in complex 3D geometries.

Live spot detection

On-line spot control – Live imaging for error correction

Spotting on a light table enables the detection of spots before they dry and hence the repair of individual missing spots

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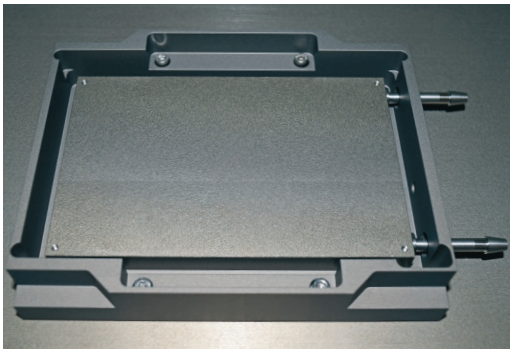
Nano-Plotter Application Note

Adapter Plates for More Efficient Microtitre Plate Cooling

Metallic inserts for the cooled microtitre plate holders

It has come to our attention that the cooled microtitre plate (MTP) holders, though accommodating practically all MTPs, are not ideal in all cases:

- Some microplates are too small so that the user must take care to move them to a specified place (e.g. the bottom right corner). Springs to fix microtitre plates are available, but they can distort flexible polypropylene plates.
- MTPs with rectangular flat bottom wells usually have sufficient contact with the flat metal plate underneath, but plates with V-shaped or round-bottom wells are not efficiently cooled because of the air surrounding the wells.
- If semi- or unskirted plates are used, the cooled air can even leave the place under the MTP, posing the risk of a temperature gradient across the plate.



A070-025 *Standard cooled microtitre plate holder. The metal plate under the MTP is even, ideal for flat-bottom wells. A microplate holder with temperature sensor is available.*

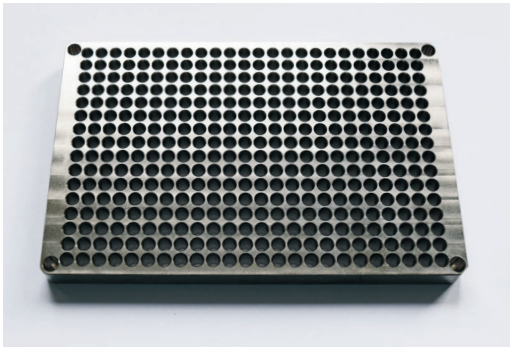
Thermocoupling via metal adapter plates

The following products are available:

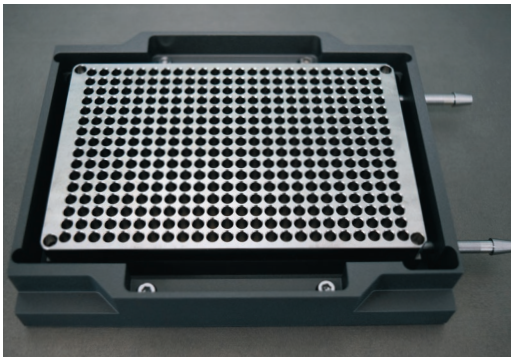
- A070-350: Adapter plate for 384 well plate
- A070-351: Adapter plate for 96 well plate

The boreholes have a flat bottom so that they accommodate both round-bottom and V-shaped wells.

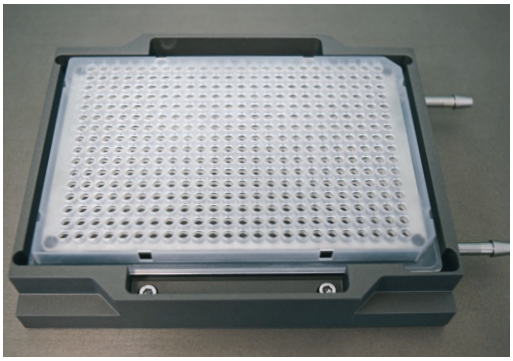
Based on the following experimental results, we recommend to use one of these adapters if your microtitre plates have round wells. Please contact us to make sure your plate fits, as different brands can have different diameters. Customization is possible.



A070-350 *Adapter plate from stainless steel for 384 well plates, before installation*



A070-350 *Adapter plate for 384 well plates from stainless steel, built into the GeSiM cooled microtitre plate holder.*



A070-350 *Microtitre plate from polypropylene with 384 V-shaped wells in the adapter plate*

Nano-Plotter
Microtitre Plate Adapters

Experimental results

We have measured the effect of the absence and presence of the adapter plates for the temperature distribution in microtitre wells. The temperature in the recirculating thermostat was set to 15 °C, where already condensation occurs at 50 – 60% relative humidity. The effect is similar at lower temperatures, except that the well temperatures are significantly higher than the bath temperature.

96 well MTP (see figure on the right):
Brand: VWR, Skirted 96 Well PCR Plate, total volume 200 µl

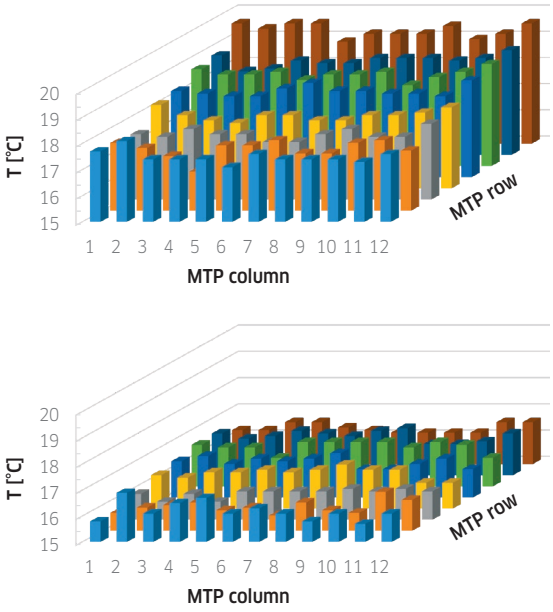
Each well was filled with 150 µl deionized (DI) water. The chiller bath was set to 15 °C.

- Without adapter plate: mean temperature 18.1 °C (CV = 3.8%), max. difference 3.1 °C
- With adapter plate: mean temperature 16.3 °C (CV = 1.8%), max. difference 1.3 °C

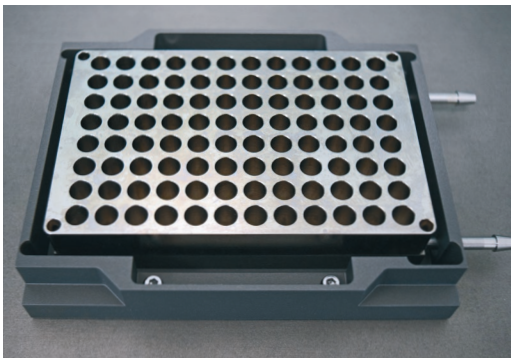
384 well MTP (data not shown):
Brand: Thermo Fisher TF-0384 PCR plate, total volume 200 µl

Each well was filled with 30 µl DI water. Chiller bath was set to 15 °C.

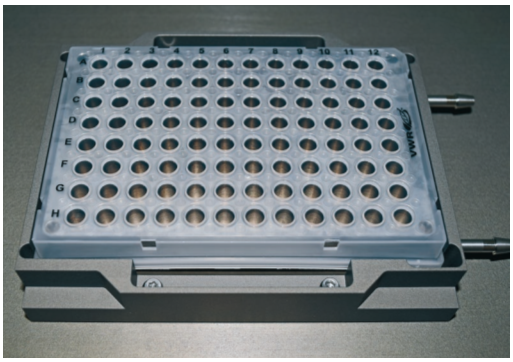
- Without adapter plate: mean temperature 18.3 °C (CV = 3.4%), max. difference 2.7 °C
- With adapter plate: mean temperature 16.3 °C (CV = 3.2%), max. difference 1.9 °C



A070-025, -351 *Temperature distribution in a 96 well plate without (top) and with (bottom) adapter plate, measured by an infrared camera. The bath temperature of the thermostat (ministat 125, Huber) was 15 °C.*



A070-351 *Adapter plate for 96 well plates from stainless steel, built into the GeSiM cooled microtitre plate holder (article number A070-025).*



A070-351 *Microtitre plate with 96 round, V-shaped wells in the adapter plate*

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Nano-Plotter Application Note

Gravimetric Determination of the Accuracy of GeSiM Piezoelectric Pipettes

Introduction

The accuracy of piezoelectric dispensers is an issue in microarray spotting. Many procedures use saturating amounts of sample, which means that binding to the surface does not depend on the volume, so constant volumes are often not necessary. Spot size and spot morphology, however, depend on the volume, and constant spot sizes result in more uniform microarrays. There are also applications in which accurate volumes are required, e.g. in pipetting/compound dilution tasks.

Here we show how GeSiM piezoelectric dispensers work in terms of volume accuracy and variation.

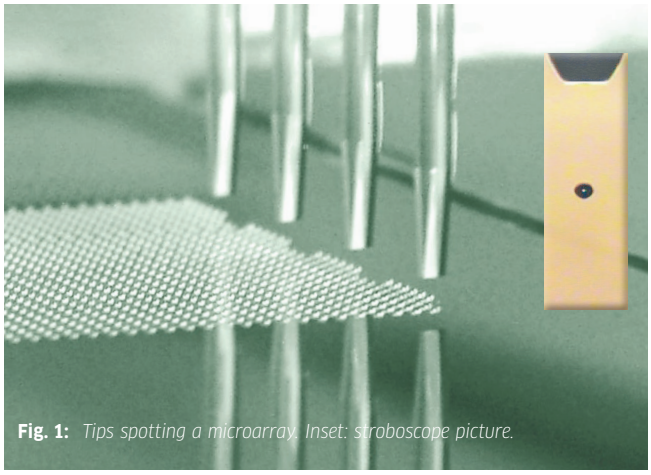

Fig. 1: *Tips spotting a microarray. Inset: stroboscope picture.*

Materials and methods

GeSiM Nano-Tips and Pico-Tips were filled with either deionized water or dimethyl sulfoxide (DMSO), and their dispensing parameters were adjusted to a defined droplet volume, determined by the GeSiM flow sensor calibrated with water. A burst of droplets was dispensed into a pre-weighed Eppendorf microcentrifuge tube and weighed in a Mettler Toledo MT5 micro-balance (precision: 1 µg, measuring range: 0 … 5100 mg). Experiments were repeated with 70% dimethyl sulfoxide (DMSO) in water.

Given the effective range of the micro-balance and the possible loss by evaporation, we figured that at least about 500 droplets are necessary for accurate weighing, corresponding to a weight of about 250 µg (250 nl) for a Nano-Tip dispensing ca. 500 pl (0.5 µg) per droplet. Smaller volumes were not measured.

In a few cases, the fluorescence for different numbers of droplets was measured. A black 384-well flat-bottom plate (BRANDplate; Merck) was filled with 50 µl of deionized water; 12 replicates each of 10 different concentrations of fluorescein (dissolved in DMSO) were dispensed into this plate by either a Nano-Tip or a Pico-Tip. The volumes were adjusted using the flow sensor. Pipetted stock solutions were 250 mM for the Pico-Tip and 50 mM for the Nano-Tip. DMSO was also used to separate sample and system liquid and to balance the DMSO content in the wells of the assay plate.

Fluorescence was measured in a Berthold LB942 TriStar 3 plate reader; filters were 485 nm (excitation) and 535 nm (emission). See also our separate application note on compound dilution.

The coefficient of variation (CV, based on standard deviation, σ, and mean value, μ: $CV = \sigma/\mu$) was calculated on the basis of the weight or the fluorescence signals of replicates.

Experimental results

Nano-Tip, gravimetric

Fig. 2 shows that the measured weight is directly proportional to the number of droplets. The slope of the regression line is 0.545 µg/droplet, at an excellent regression coefficient of 0.9999. Given the density of water of 1 g/ml, the resulting volume per spot of 545 pl is very close to the volume determined by the flow sensor (540 pl), only off by 0.9%.

Fig. 2 shows that at lower droplet counts, the deviation from the theoretical value is significant, probably due to evaporation. At higher droplet counts (above ca. 1000 – 2000), this error mostly disappears. The coefficient of variation for the measurements is very low and never exceeds 1%. On the one hand this is due to the higher volumes that were dispensed, but also because inaccuracies average out at high numbers, a fact we have already observed in the early days of the GeSiM nano-pipettes.

Gravimetric measurements with 70% DMSO gave comparable results, with a slope of 0.453 µg/droplet versus a theoretical droplet volume of 470 pl (deviation: –3.7%). This deviation to theoretical values was also visible at high droplet counts (not shown).

Nano-Plotter

Accuracy of GeSiM Piezo Pipettes

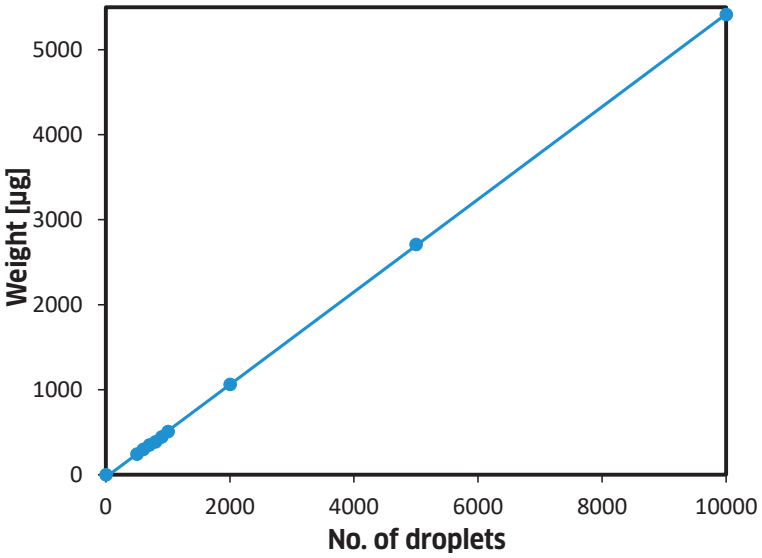

Fig. 2: *Weight versus number of droplets for water, dispensed by a Nano-Tip. As the weight in µg equals the volume in nl, the slope (0.545) yields the volume of a droplet in nl, which is very close, within 0.9%, to the value measured by the flow sensor (0.54 nl).*

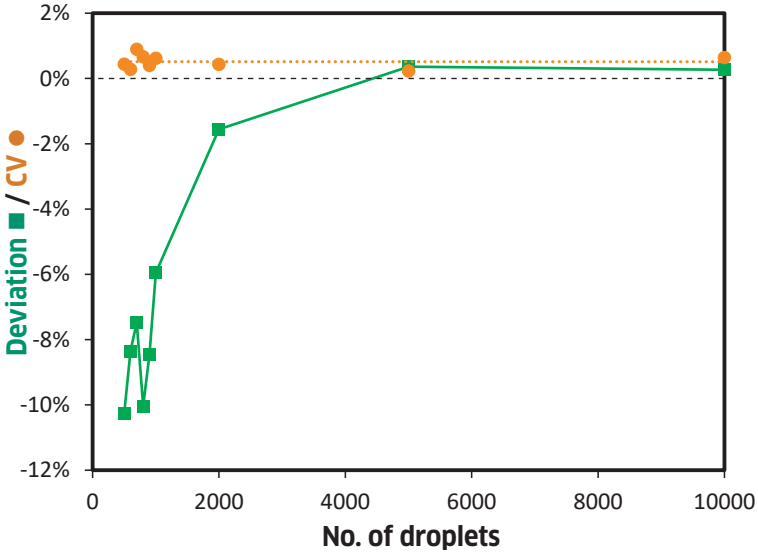

Fig. 3: *Deviation of the measured weight from the value determined by the flow sensor, and CV of the droplet weight measured by gravimetry, for droplet numbers between 500 and 10000 dispensed by a Nano-Tip.*

Nano-Tip and Pico-Tip, fluorescence measurements

To address the situation for small bursts of droplets, we measured the fluorescence of diluted solutions. Spotting parameters were adjusted so that the piezo tips dispensed 600 pl (Nano-Tip), 100 pl (Pico-Tip) and 50 pl (Pico-Tip) into a constant volume of water (50 µl).

We show here the range where the curves are predominantly linear, which is the case for dispensed volumes of about 1 – 10 droplets (Nano-Tip 600 pl, Pico-Tip 100 pl) and about 1 – 20 droplets (Pico-Tip, 50 pl droplets). As the curve determined by gravimetry is linear for higher dispensed volumes, it is likely that the non-linearity of the fluorescence signal stems from the inner filter effect. CVs determined here were around or smaller than 5% (see separate app note).

Conclusion

From dilution experiments with fluorophores with volumes from 1 to about 20 droplets and gravimetric measurements of higher volumes (> 1000 droplets), we conclude that the volume dispensed by GeSiM piezoelectric pipettes depends linearly on the droplet number across a wide range of droplets, with low CV numbers and deviations to theoretical volumes that are smaller than a few percent.

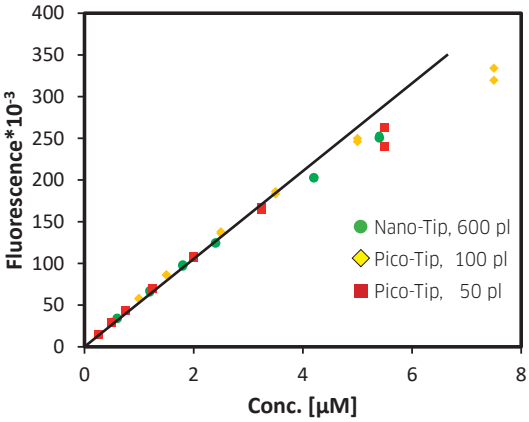

Fig. 4: *Fluorescence vs. final concentration for different piezo pipettes. Numbers at the tips indicate the volume per droplet in picolitres.*

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Nano-Plotter Application Note

Compound Dilution and CVs of GeSiM Piezoelectric Pipettes

Introduction

The Nano-Plotter is mostly used for microarray production. Here we describe how it can be used for *pipetting*.

The method described here is also called direct titration, as opposed to serial dilution, as you fill an amount of buffer or solvent into wells, and the Nano-Plotter dispenses different volumes of stock solution into these wells to obtain all different dilutions of your compound.

As different numbers of droplets (down to a single one) are dispensed, these experiments also give you an idea of the accuracy of the piezo pipettes.

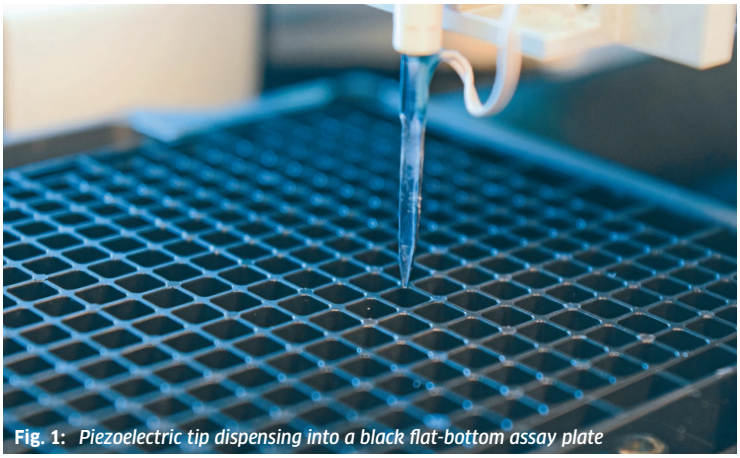

Fig. 1: *Piezoelectric tip dispensing into a black flat-bottom assay plate*

Prerequisites

- Copy the configuration programme, *CompoundDilution.exe*, and *CompoundDilution.npl* to the NPC16 *Prog* folder.
- On the work plate, define a **source plate** named *STOCK* (for stock solutions, buffers/solvents) and a **target plate** named *ASSAY*.

Starting *CompoundDilution* in the NPC16 Run mode initiates the external *.exe* programme, showing the set-up dialogue in Fig. 2. →

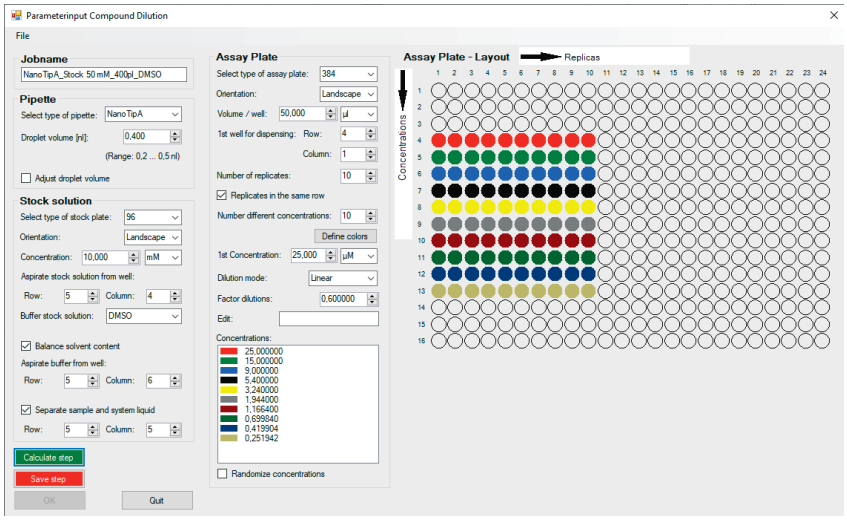

Fig. 2: *Start dialogue*

For the *STOCK* plate you enter:

- Droplet volume of the pipette
- Type and orientation of the plate
- Concentration of the stock solution
- Solvent/buffer used in the stock solution and its location in the plate

'Buffer solvent content' means that the volume dispensed into the wells is adjusted with solvent to obtain constant total volumes; you can also separate sample and system fluid in the piezo pipette by a plug of buffer/solvent. For the *ASSAY* plate you define:

- Type and orientation of the plate
- Volume of liquid (buffer) that is placed in the wells
- Well into which the first concentration shall be pipetted
- Number of replicates (identical samples, usually in a row)
- Number of dilution steps and dilution factor
- You also set whether dilution from row to row is linear or logarithmic; it can also be manually determined when dilution mode is 'free'.
- The 'File' menu at the top lets you load and save procedures.
- [Calculate step] illustrates the layout of the assay plate and displays the calculated concentrations. The button turns green.
- [Save step] transfers data to the Nano-Plotter software. Do this right before pipetting.

[OK] starts pipetting. The minimum number of droplets dispensed is one, so dilution may be inaccurate for some conditions.

The dilution is done row by row, but the layout can be randomized to reduce systematic errors that mainly stem from evaporation starting along the edges.

Materials and methods

The assay plate, a black 384-well flat-bottom plate (BRANDplate; Merck), was initially filled with 50 µl of deionized water; 12 replicates each of 10 different concentrations of fluorescein (dissolved in DMSO) were then dispensed into this plate by either a Nano-Tip, a Nano-Tip A, a Pico-Tip or a solenoid valve dispenser. DMSO was also used to separate sample and system liquid and to balance the DMSO content in the wells of the assay plate.

After spotting, the fluorescence of the wells was measured in a Berthold LB942 TriStar 3 plate reader. Optical filters were 485 nm (excitation) and 535 nm (emission). Measurement time per well was 0.1 s at an excitation voltage of 600 V. The coefficient of variation (CV, based on the standard deviation, σ, and the mean value, µ: CV = σ/µ) was calculated on the basis of the fluorescence signals of the replicates.

Experimental results

Nano-Tip (600 pl) and Nano-Tip A (300 pl)

Spotting parameters of a Nano-Tip were adjusted to droplets of 600 pl, aided by the GeSiM flow sensor. Likewise, a Nano-Tip A was brought to 300 pl. The stock solution was 50 mM fluorescein. Ten different concentrations were generated in the assay plate, leading to a dispensing volume per well between 0.6 (1 droplet) and 25.2 nl (42 droplets) for the Nano-Tip; for the Nano-Tip A it was between 0.3 (1 droplet) and 20.1 nl (67 droplets).

Pico-Tip (100 and 50 pl)

Pico-Tip spotting parameters were adjusted to droplet sizes of either 100 or 50 pl. The stock concentration was 250 mM. Six different concentrations were generated. For 100 pl droplets, this led to dispensing volumes per well between 0.1 (1 droplet) and 5 nl (50 droplets). For 50 pl droplets, this resulted in dispensing volumes per well between 0.05 (1 droplet) and 5 nl (100 droplets). Results for the tips are shown on the right.

Solenoid valve dispenser (30 nl)

A solenoid valve dispenser was adjusted to 30 nl drop volume of 30 nl. One to 27 drops (30 to 810 nl) of a 2.5 mM stock solution of fluorescein were dispensed into 50 µl.

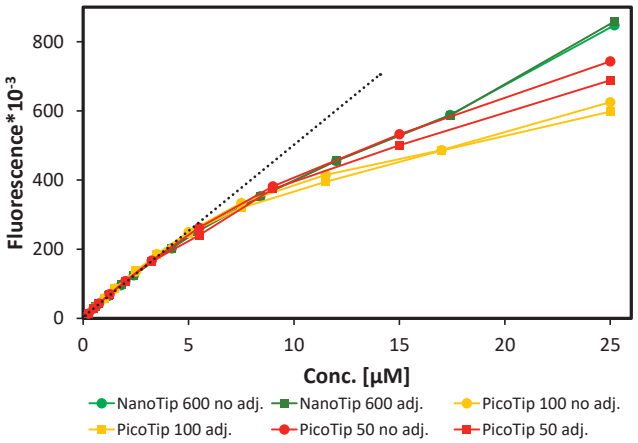

Fig. 3: *Fluorescence vs. concentration for different piezo pipettes, without ("no adj.", ●) and with ("adj.", ■) volume compensation. Numbers indicate the volume per droplet in pl. Initial slopes are perfectly linear (dotted line).*

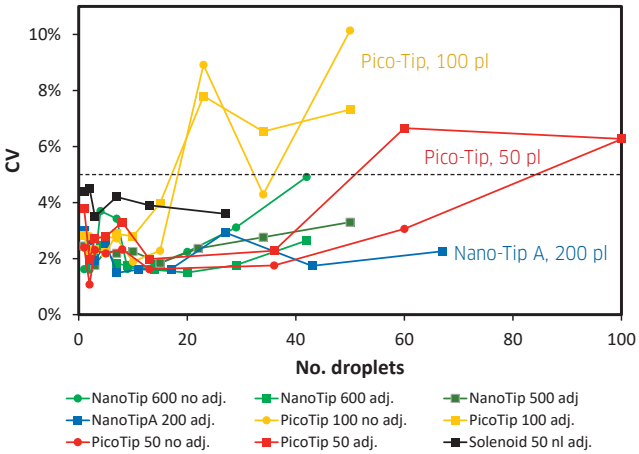

Fig. 4: *CVs for various pipette types, as above. Except for the solenoid valve dispenser, numbers indicates the volume per droplet in pl.*

Conclusion

Compound dilution works well in the range between one and about 100 droplets. As the gravimetric determination of dispensed volumes shows a perfectly linear behaviour (see other app note), we attribute the non-linearity of the fluorescence curves at high concentrations to the inner filter effect. CV values lie between ca. 1 and 5% across the whole range and hardly exceed 10%.

We hence conclude that the Nano-Plotter can be used for accurate compound dilution.

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Nano-Plotter Application Note

Line Printing for the Manufacturing of Lateral Flow Assays

A common spotting application is the generation of lines, mainly for the fabrication of **lateral flow tests**. Lines are printed onto membranes that are then cut in strips and packaged. The GeSiM Nano-Plotter has a built-in line printing function: the head accelerates to the start position and then spots at constant speed and droplet frequency before it decelerates.

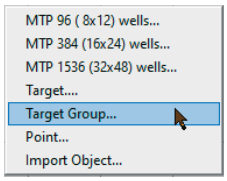
Prerequisites

- Spotting programme *OneTip_LinePrinting*, repair programme *OneTip_LinePrinting_Repair.npl*, *OneTip_LinePrinting_WashSampleCheck.inc* and a certain version of *TransferSimMultiPlates*
- *One* piezoelectric tip, or a solenoid valve dispenser for broad lines. Lines are printed in the Y-direction.
- One or more membranes, fixed on the target holder using tape or vacuum
- Optional: a large enough container with extra wash solution

The work plate

Work plate objects are defined in the 'edit' mode as follows:

- Source microplate, *MTP_Sys* (determine position before printing)
- Target group named *Membranes*, see below
- 'Dummy' microtitre plate, *ExWash*, if harsher wash conditions are needed



Create a new target group via the context menu.

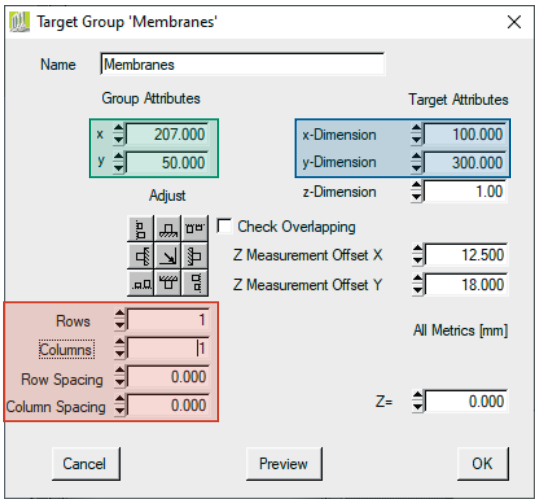
Define a new target group, *Membranes*, and take care of these properties:

- Number of targets (rows/columns) and the spacing in between
- Position of the target group (bottom left corner)
- Target size (check that all targets can be accessed)

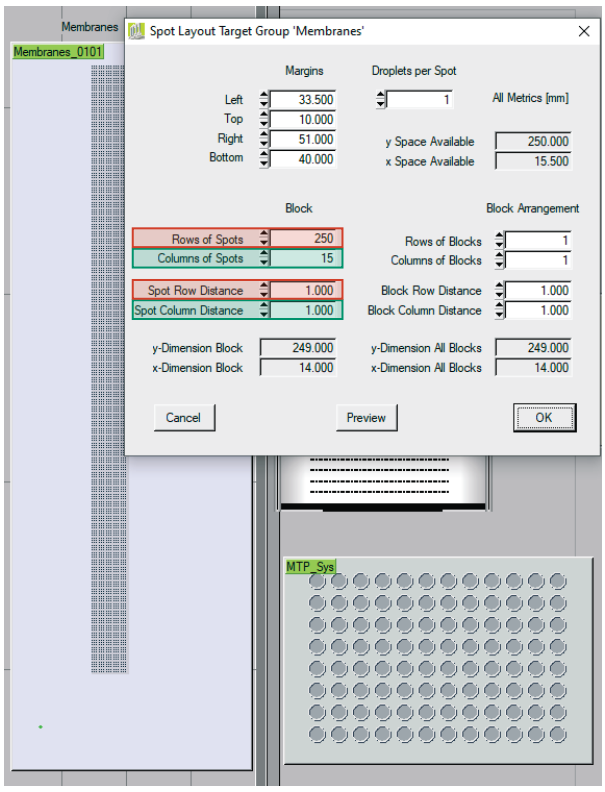
Right-click on the target group to edit its spot layout ('New Spot Layout… → Rows Columns…' or 'Edit Spot Layout…'). In the upcoming window (see right picture), enter these parameters:

- Number of lines (= spot columns) and their distance
- Length of the lines, defined by the number of spot rows and the spot row distance. Lines will be printed from the first spot down to the last. The spacing of the spots is given by speed and droplet frequency. (Leave room for acceleration/deceleration.)

Measure the Z-height of all targets and check the position of the MTP.



'Properties' window for the target group Membranes (typical values)



Spot layout definition for a membrane object

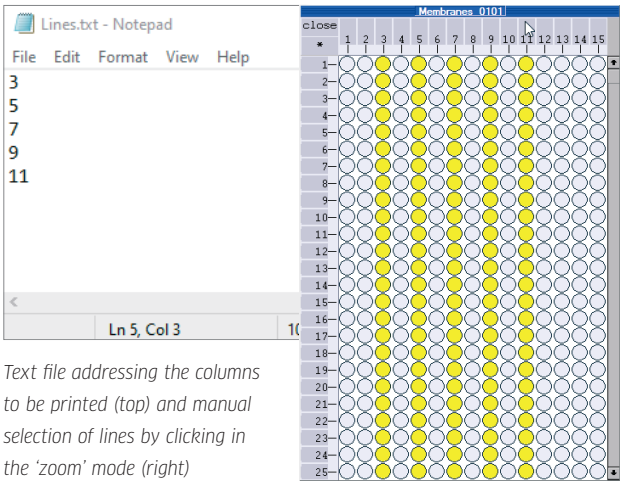
Nano-Plotter
Line Printing

Before you begin

Decide how to tell the programme which columns (lines) of the microarrays on the targets shall be printed, either using a file or by manually clicking lines in an array.

- To read in data, prepare a text file in which each number in a line of text represents a column of the array.
- Or zoom into an array block of the target (context menu) and mark the lines to be printed, e.g. by clicking the column number above the microarray grid.

In both cases shown on the right, columns (lines) 3, 5, 7, 9 and 11 of the microarray grid will be printed as lines.



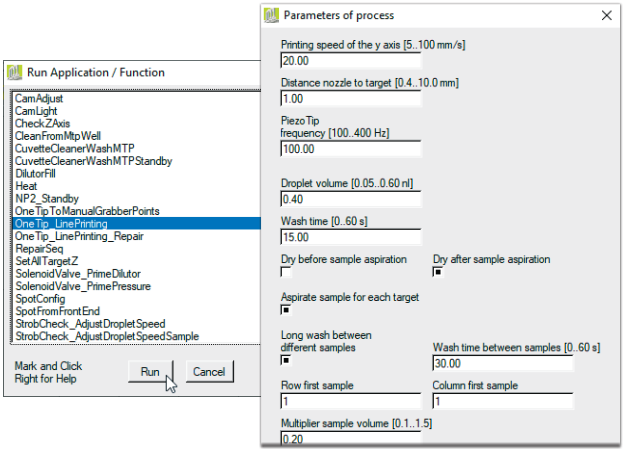
Text file addressing the columns to be printed (top) and manual selection of lines by clicking in the 'zoom' mode (right)

Spotting

Enter the run mode and open the list of spotting programmes (or 'Program → Run NPL application…'). Press [Run] or double-click to start. (If there are too many tips, you'll get an error message.)

- To read data from a file, say [Yes].
- If you click [No], the lines must already be selected (see above).

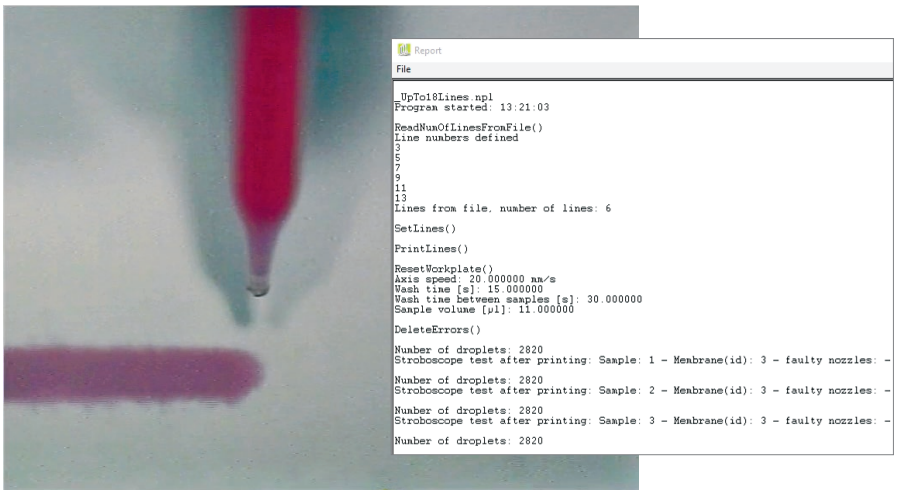
Enter parameters for the line printing process, especially speed and droplet frequency, and start by clicking [OK].



Programme selection (left) and line printing parameters (right). If an ExWash object exists, parameters for the extra wash are also shown here.

- If there is a head camera looking at the nozzles and you selected 'Show head camera window when printing', you see a live picture of the print process (here using a GeSiM solenoid valve dispenser).
- By opening the 'report' window, you can read what is currently happening. (You can save this report later.)

The next line will be printed with the next sample in the source plate and this goes on until all columns are finished. Errors are corrected by running *OneTip_LinePrinting_Repair*.



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Nano-Plotter Application Note

Bitmap Converter – Print Images with the Nano-Plotter

The **Bitmap Converter** converts bitmap files (.bmp , .jpg, .png, .tiff etc.) so that they can be printed on a Nano-Plotter using the programme system SpotFrontEnd/SpotFromFrontEnd (SFE/SFFE).

Resulting pixels are either printable or non-printable. The Bitmap Converter lets you change spot distance, size or resolution, but you cannot change the resolution while maintaining the overall size. If the resulting pattern does not fit on the target or contains too many spots, resize the bitmap in an external graphic programme, e.g. GIMP or Photoshop.

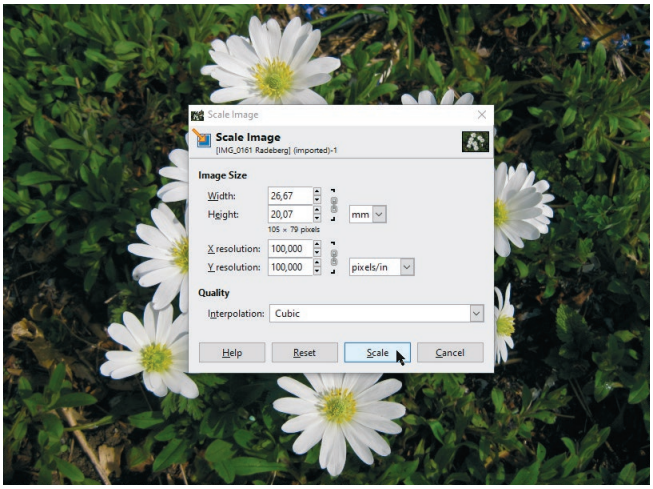
Avoid too many points by using a small resolution. Also try to change/separate colours, brightness/contrast and other settings or erase features or crop the picture to enhance the picture for printing.

Prerequisites

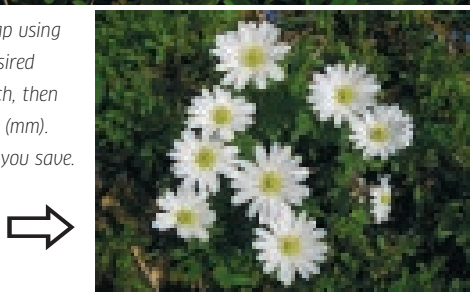
- The spotting system SpotFrontEnd/SpotFromFrontEnd that is bundled with image recognition, but can also be obtained if you don't have a video microscope.
- *BitmapConverter.msi* and *setup.exe*
- Microsoft Visual C++ Redistributable (*vcredist_x86.exe*)
- Initialise the setup and follow the instructions. When you are requested to install the Visual C++ runtime, grant it or repair the existing one.

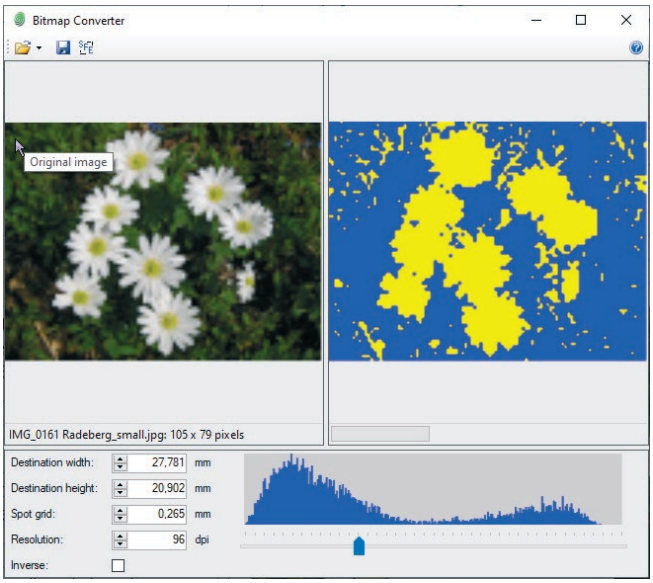
Run the Bitmap Converter

- Start the Bitmap Converter in the Windows Start Menu and load your bitmap.
- The bitmap is displayed in the left panel of the window. The right panel shows the converted bitmap with filled (blue) and empty pixels (yellow).
- To adapt the bitmap to your target, you can change either the size ('Destination width/height') or the spot grid or the resolution in dpi. Changing one parameter will automatically change all other parameters. So to reduce the resolution, you must use an external graphic programme. Make sure the bitmap fits on the designated target and the spotting distance is not too small.
- The slider bar under the images lets you change the threshold between bright and dark, i.e. between pixels that are printed and those that are not printed. On the rear page, the slider is moved further to the right to include more pixels.
- The 'Inverse' check box inverts dark and bright pixels (remember, active pixels are blue).



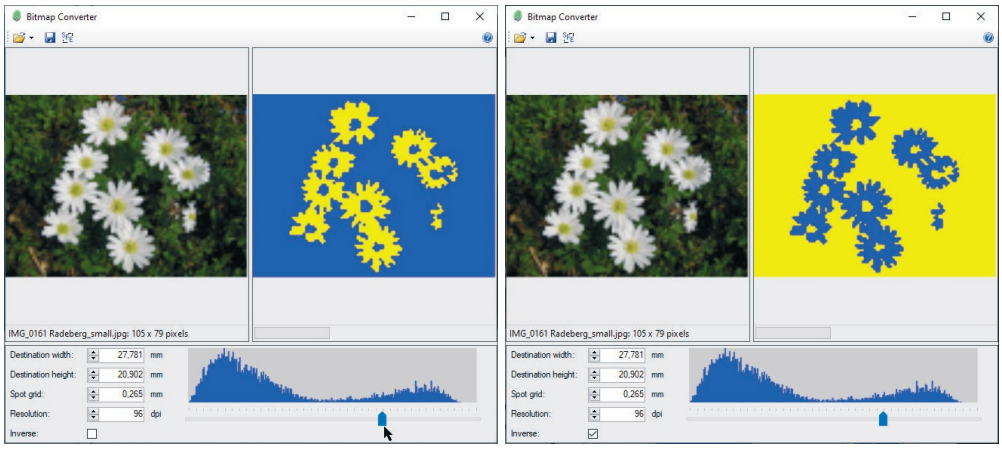
How to resize a bitmap using GIMP. First set the desired resolution in pixels/inch, then the size of the picture (mm). Check settings before you save.





Bitmap loaded. On the right the result of the conversion is shown. A histogram with pixel intensities and a slider bar is displayed at the bottom.

Nano-Plotter
Bitmap Converter

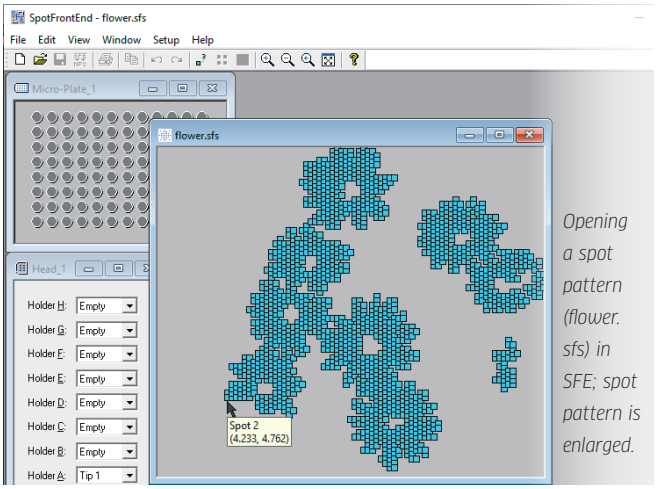


Changing the threshold to include more printed points (left) and inverted picture (right)

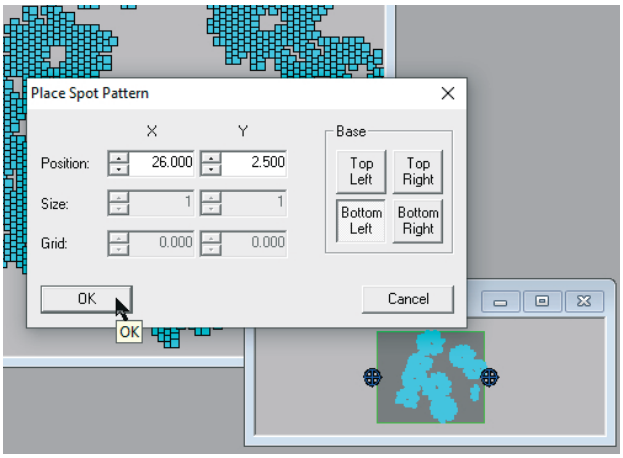
- By clicking the [SFE] button, export the data to files that are used by SFE: first create a target with the size of the canvas (.sft), then a spot pattern (.sfs). The points in the spot pattern file are numbered column by column, starting left.
- Or save the pixel positions as a list file (.lst) by clicking the disk button (for programmers).

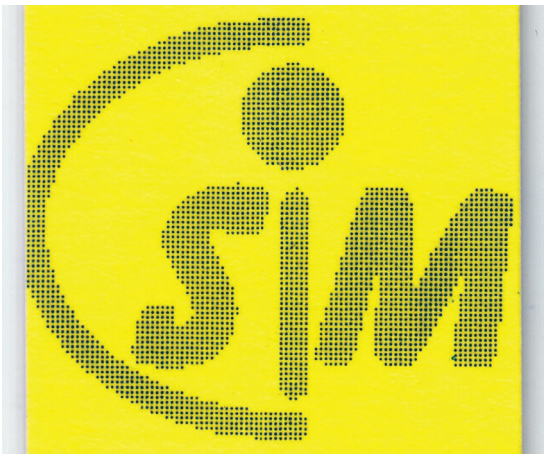
Print your bitmap with the Nano-Plotter

- Open SpotFrontEnd (SFE). Define MTP type and pipette head configuration, exactly as used in the Nano-Plotter.
- Open the spot pattern exported by the Bitmap Converter.
- Create a new target and insert the spot pattern at a defined X/Y position, or use the target file created by the Bitmap Converter.
- Renumber the first point, if necessary. Maybe add another spot pattern, e.g. for a second 'colour'.
- To work without alignment marks and video microscope, set the aligner type to 'none'. Otherwise define alignment marks (manual, dot array, custom) as usual.
- Prepare a spot plan by assigning sample wells to spot numbers and export the spot plan to SFFE for printing.
- Print the bitmap using SFFE on your Nano-Plotter.



Opening a spot pattern (flower.sfs) in SFE; spot pattern is enlarged.





Print example: GeSiM logo on water-sensitive paper; width of the paper: 26 mm.

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Nano-Plotter Application Note

Ink-Jet Printing of Commercial Conductive Inks Using GeSiM Piezo Tips

Introduction

Printed electronics is a hot topic in microelectronics where electrodes can serve e.g. as actuators and sensors on foils, but also in biology where electronic actuators can be incorporated into 3D-printed bioinks. Here we investigated whether the GeSiM Nano-Plotter can be used for this goal.

Materials and methods

Nano-silver inks and, for comparison, water were printed with a Nano-Plotter 2.1/E using these piezo dispensers (in certain cases heatable):

- Pico-Tip (normal droplet size at 70 V: 60 pl)
- Nano-Tip A (normal droplet size at 80 V: 250 pl)
- Nano-Tip (normal droplet size at 70 V: 350 pl)

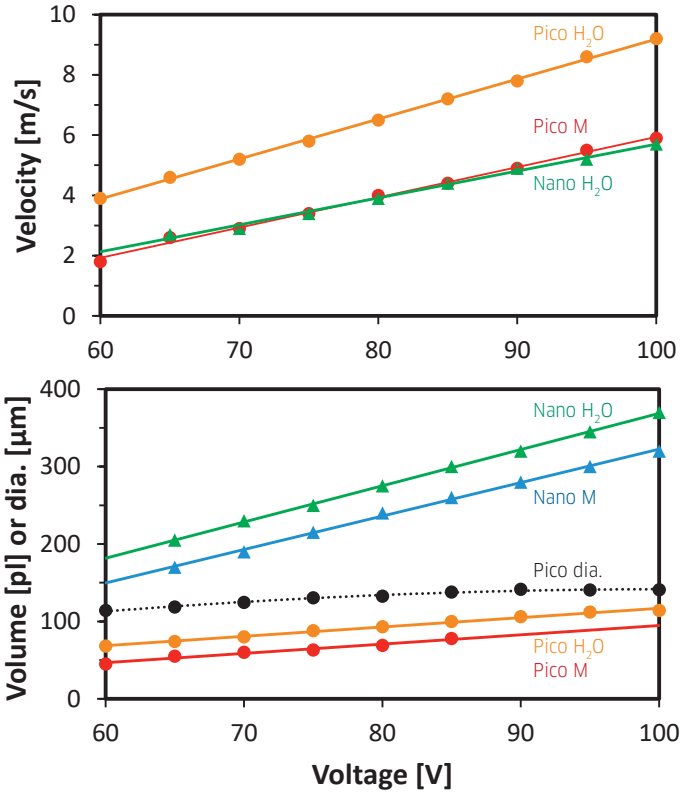

Fig. 2: *Spotting parameters for Nano-Tip A and Pico-Tip at different voltages. Spot diameters for Pico-Tip were measured on PET surfaces. The following abbreviations are used: Pico, Nano = Pico-Tip or Nano-Tip A; H_2O, M = spotting with water or Metalon; dia. = diameter of Pico-Tip spots with Metalon.*

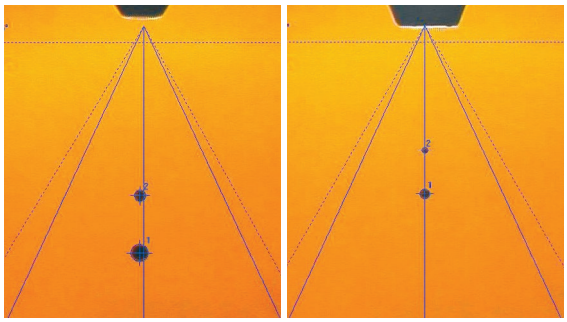

Fig. 1: *Stroboscope images of a Nano-Tip A (left) and a Pico-Tip (right) dispensing NovaCentrix Metalon ink*

Inks were diluted with organic solvents or water according to manufacturers' instructions and aspirated from a microtitre plate. CAD drawings were converted to transfer lists that were printed with TransferSim using 'fast list transfer'. Solvent-based inks required an air gap to separate them from the system water. Pulse width for all pipettes was 90 µs at 100 Hz. The toxic inks had to be handled with care.

Curing was done as recommended by drying, irradiation, or heating at temperatures between 60 and 200 °C for 20 to 120 min. Dispensing of the water-based Metalon ink was mostly done onto Novele foil, a PET derivative. Curing of this ink was done by short illumination (30 µs) with high power pulsed light (peak intensity at 400 – 600 nm) in a NovaCentrix PulseForge device (www.novacentrix.com), in cooperation with merconics GmbH & Co. KG, Radeberg.

Results

The results are summarized in the table on the next page:

- All inks, with and without air gap, could be dispensed with GeSiM's piezo tips, sometimes after slight dilution with triethylene glycol monomethyl ether (TGME), water or other solvents
- The dispensing behaviour of all inks was similar.
- Droplet volumes and speeds increase at higher piezo voltages. Due to their viscosity, droplet volumes and speeds of the inks were smaller than those of water, but this only marginally affected their printability.
- Smart Jet L, Flow Metal SR7500-1 and Dycotec: the material stuck to the outside of pipettes.

Name	Viscosity [mPa·s]	Solid content [wt%]	Type	Dilution / dispensing
GenesInk Smart Jet L S-CS01130	13	15–25	Alcohol-based	Works undiluted or 1+1 diluted with "CleanJet"
Bando Flow Metal SR7500-1		50–55	Solvent-based	Works without dilution
Bando Flow Metal SW1020		35–45	Water+alcohol-based	Stably dispensed either undiluted (but with elongated droplets) or 1+1 diluted with DI water
Dycotec DM-SIJ-3200	10–18	37–40	Solvent-based	Dissolved at 60-80 °C, dispensed at 100 °C; works undiluted or after 1+1 dilution with TGME
Sigma Aldrich Silverjet ITO DGP-10TE-20C	10–18	30–35	Solvent-based	Stably dispensed after 1+1 dilution with TGME
Sigma Aldrich Silverjet DGP-40LT-15C	10–18	30–35	Solvent-based	Dissolved at 120-150 °C; works undiluted or 1+1 diluted with TGME
NovaCentrix Metalon JS-B25P	3–5	25	Water-based	Dispensed onto PET and Novele, with *PulseForge* curing

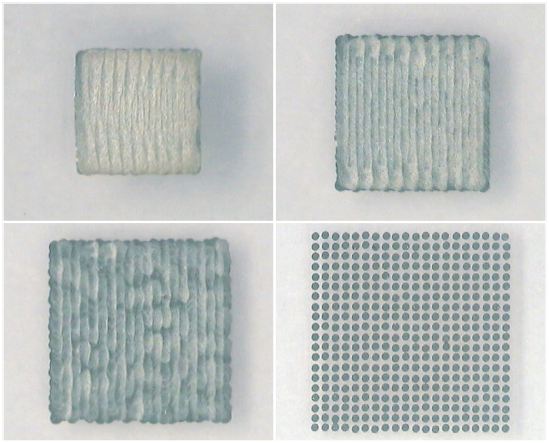

Fig. 3: *Metalon arrays of 20×20 spots printed with a Pico-Tip. Droplet distances were 40, 50, 60 and 110 µm (different magnification used for 110 µm).*

- Wetting of gold surfaces was often not ideal; this was also true for silicone even when cleaned with acetone. On the other hand, plasma-treated silicone resulted in spreading of the spots.
- The resistance of a bridge of Bando Flow Metal SW 1020 between conducting paths was 0.2 Ω.
- Generally microarrays on paper did not look nice (not shown), but other materials (e.g. PET for Metalon) led to even prints.

Effects were studied in detail for NovaCentrix Metalon. Droplet patterns in the stroboscope looked normal, and the Nano-Plotter enabled manufacturing of printed electronics without gaps, ideally on the recommended Novele foil. Without curing, this ink was an insulator whereas brief illumination with high power pulsed UV light instantly led to a conducting layer without affecting the underlying substrate.

Summary

We show here that the GeSiM Nano-Plotter not only prints microarrays, but also larger areas in any geometry in which the spots contact each other, of course dependent on the nature of the ink and the surface. Paper, for instance, is not well suited, nor is silicone (neither cleaned with acetone nor plasma-oxidised). In many cases PET is ideal, a flexible substrate.

So if you own a Nano-Plotter microarray spotter, you don't have to resort to instruments that are especially designed for printed electronics, but can continue using it (though not quite at the same speed and resolution).

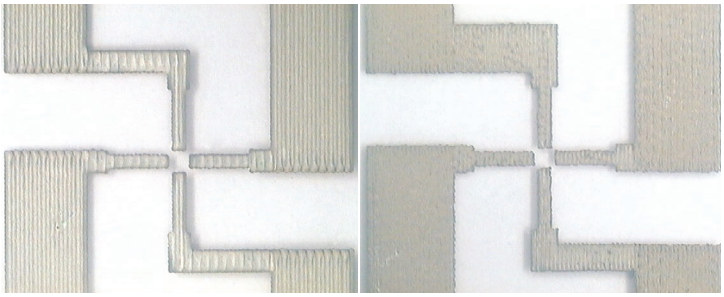

Fig. 4: *Microelectrodes from Metalon, printed with a Nano-Tip (left) and a Pico-Tip (right), with subsequent photonic curing. The final structure is a dielectrophoretic field cage made of four electrodes in the centre; the outer regions are contact pads.*

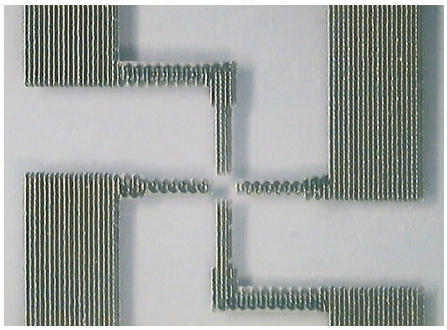

Fig. 5: *Microelectrodes printed with Bando FlowMetal SW1020 (Nano-Tip A) and cured at 150 °C for 30 min. In this case the printed electrode structure is non-overlapping.*

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Nano-Plotter Application Note

Manufacturing of Micro-Needle Arrays

Why micro-needle arrays?

Micro-needle arrays are regularly arranged groups of small spikes in the range of a millimetre or less. Various types (solid, coated, dissolving, hollow) made of different materials exist. They are increasingly popular because they can be applied for the subcutaneous delivery of pharmacologically active ingredients or vaccines, as they painlessly breach the outer skin layer, and are thus an attractive alternative to injection using hypodermic needles and allow self-administration.

Different approaches have been used to deliver drugs by micro-needle (MN) arrays: bioresorbable polymers blended with deliverables or non-soluble needles, with reservoirs for active ingredients or just coated with them.

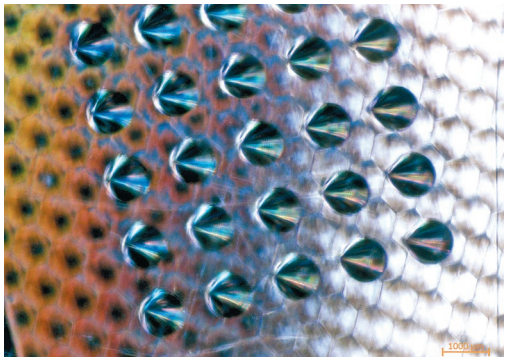
Many ways exist to produce MN arrays, among them 3D stereolithography (SLA) printing, micromilling and injection moulding, all of which have their pros and cons. We chose to employ **nanoimprint lithography (NIL)**, as it is reproducible, modestly expensive and combines the benefits of the other methods:

- Affordable instrumentation
- Low operational costs
- Small design features down to 1 µm that aid in drug delivery
- Quick manufacturing (approx. 5 min per MN array)
- Wide range of printable materials

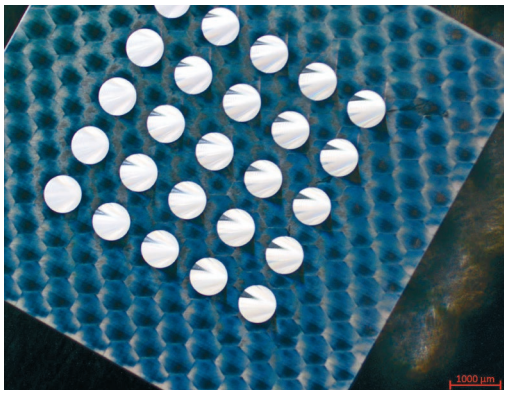
Technically, a stamp made of PDMS or (better) perfluorinated polymer (PFPE) is used along with a UV-curable or thermomelting resin in which the stamp is immersed during the imprinting process.

The **GeSiM microcontact printers**, e.g. the µCP4.x, are ideally suited for this task because they apply vacuum during printing so that each cavity is completely filled with resin, allowing the reliable formation of needles of **high aspect ratio**. This process is described in detail in another application note. Please inquire if you'd like to know more about our instruments for microcontact printing and NIL or if you would like to have your Teflon-coated stamp masters manufactured by GeSiM.

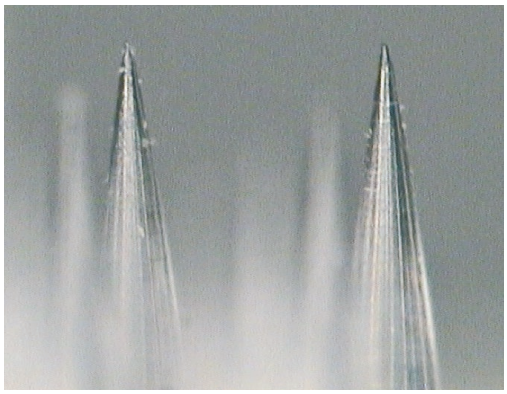
In addition, the outstanding spatial resolution and reproducibility of GeSiM's NIL technology enables the fabrication of micro-needles also with special features such as grooves, indentations and channels into which drug solutions can be deposited.



Micro-needle master, manufactured by 2-photon-lithography on top of a silicon substrate. This is a service provided for our µCP customers.

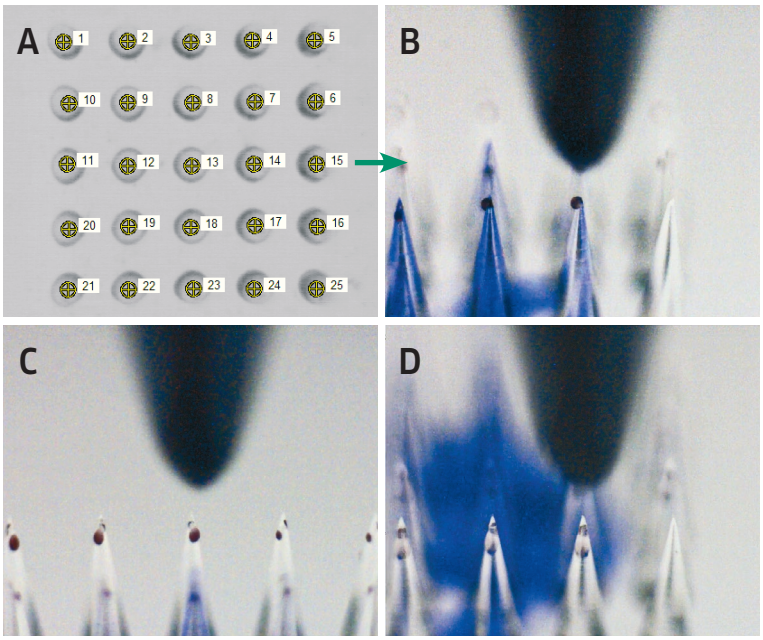


PDMS stamp, bottom view, with conical cavities. This disposable print tool is made on your lab bench.

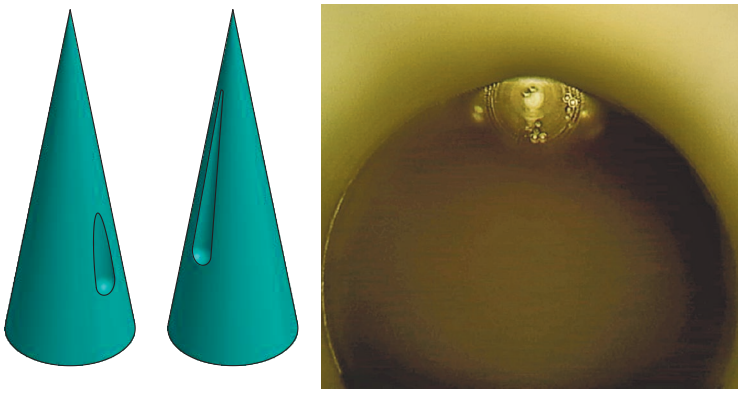


Printed MN array prepared from NOA63 photoresist (Norland). Here: height 1.2 mm, base diameter 0.4 mm.

Nano-Plotter
Micro-Needle Arrays



Piezo spotted droplets on a micro-needle array; dimensions see below. A, depiction of the points onto which sample will be dispensed, after image recognition of the 'dot array'. B, spotting of the first spot (containing PBS buffer and black ink), filmed with a video microscope. (The extra fine dots at the tips are reflections.) C, spotting of a second drop onto the same MN array (pipette moving from left to right). Note the increased spot size on the left tips. D, micro-needles were plasma-treated to produce a hydrophilic surface, demonstrating the spread and improved adhesion of drops on such a surface.



Needle design with spherical groove (diameter 80 µm, volume about one nanolitre), shown from two sides. The conical needle has a height of 1200 µm and a base diameter of 400 µm; in the examples above, the pitch was 570 µm. Right picture, single needle viewed from the top. In the lateral groove you can see 8 µm beads that were entrapped after liquid deposition by a Nano-Plotter piezo pipette (needles were tilted for spotting). Needles with a length of 1000 and a diameter of 600 µm were also tested (not shown).

Piezoelectric coating of micro-needle arrays using the GeSiM Nano-Plotter

Piezoelectric nanolitre pipetting is useful to tether (bio)molecules to the needle surface. The Nano-Plotter is ideally suited for this because it not only is accurate and contact-free, but also uses automatic image processing with our *SpotFrontEnd* programme system to exactly place liquids in the sub-nanolitre or nanolitre range onto arbitrary spot patterns. Higher volumes can be achieved by either dispensing more droplets per spot or by using our solenoid valve dispensers that can eject drops of 20 nl or more.

- A microscope on the pipette head detects the positions of the micro-needle arrays, as follows:
- First, round objects of a certain size are identified and the position of two corners of this 'dot array' and hence the position and rotation of the object exactly determined. This step is repeated for all objects loaded on the substrate tray.
- After washing and sample uptake, the droplet pattern in a stroboscope is analysed, and if this is successful, the system advances to the next step.
- A test spot with sample is printed on a yellow water-sensitive paper to measure the droplet deflection. This guarantees high positional accuracy.
- The micro-needle array is coated according to the geometry of the array and the spot plan that links samples to array positions. The previously determined deviation of the spot position from the theoretical position is taken into account.
- Missing spots due to errors in the stroboscope or the yellow paper test can be repaired afterwards.

Conclusion

We show here that it is not only possible to create micro-needle arrays with high aspect ratio using NIL on our µCP4.x, but also that fluid samples can be accurately deposited onto these needles using our microarray spotter, Nano-Plotter NP2.1, with automatic target recognition.

Of course, the Nano-Plotter can also print on micro-needle arrays made by other means, e.g. stereolithography (SLA).

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Nano-Plotter Application Note

Ink-Jet Printing of PDMS Solutions Using GeSiM Piezo Tips

Introduction

Polydimethylsiloxane (PDMS) is a widely used silicone elastomer with interesting properties such as low cost, biocompatibility and optical transparency.

Ink-jet printing technology can be used for additive manufacturing, as it allows the deposition of small amounts of material at specified positions anywhere on a substrate. Dispensing of several layers on top of each other eventually results in complex 3D structures.

Whether polymers can be printed using piezoelectric dispensers depends on properties of the material such as viscosity and surface tension. The permissible viscosity of piezo dispensers is often limited to a few tens of mPa·s or less, depending on the dispenser design. Heating would reduce sample viscosity, but is often undesired. It has been shown recently (see ref.) that dilution with octyl acetate (OA) can lower the viscosity of PDMS to such an extent that it can be printed at room temperature using standard ink-jet dispensers.

Here we show how GeSiM's piezoelectric tips can be used to dispense PDMS inks at room temperature.

Materials and methods

PDMS (Sylgard 184, Dow) was mixed at a ratio of 10:1 (v/v) with curing agent and degassed before printing. Octyl acetate (= octyl ethanoate, Sigma-Aldrich) was used to dilute this PDMS mixture.

GeSiM's microarrayer, Nano-Plotter NP2.1/E, was equipped with different types of GeSiM piezo tips. Tips were connected to a PTFE tubing system containing a syringe pump with 3-way valve (diluter). PDMS ink was aspirated from a micro-well plate, and the solution was separated from the system liquid (= de-ionized water) by using an air gap and a buffer of extra solvent. Droplet volumes were measured with the GeSiM flow sensor while dispensing was controlled in a stroboscope.

PDMS arrays were dispensed using rectangular voltage pulses onto standard 1×3 inch ((25.4×76) mm²) glass slides whose surface had been modified by exposure to silane vapour. The contact angle of water after this treatment is usually 90°. PDMS spots were dried and cured in an oven at 120 °C for 30 minutes and the resulting spot diameters measured in a calibrated microscope.

Experimental results

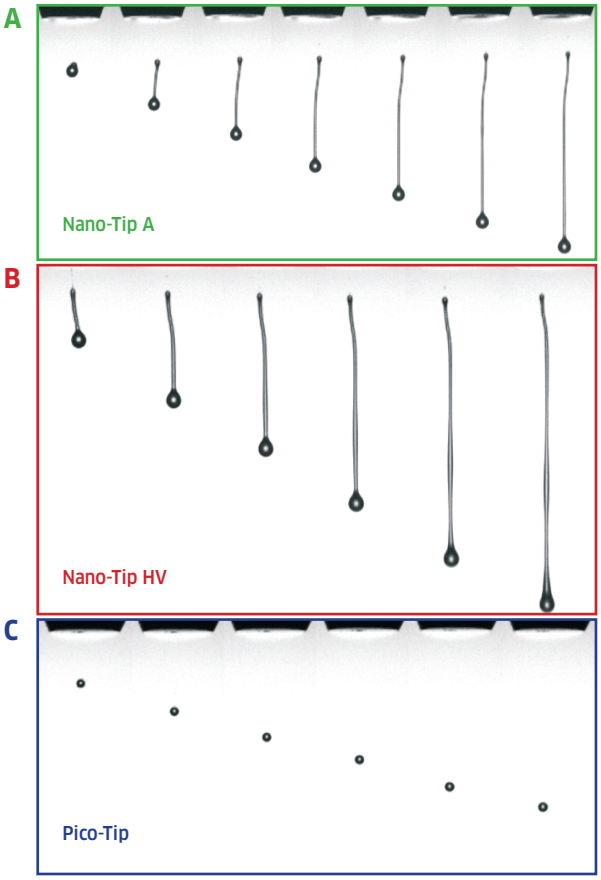

Fig. 1: *Droplet shapes, measured 250 µs after triggering of the piezo, for different dispensers (same scale).* ***(A) Nano-Tip A*** *for a PDMS:OA ratio of 1:3 and piezo voltages between 60 and 120 V,* ***(B) Nano-Tip HV*** *for a 1:3 PDMS–OA solution and voltages between 60 and 120 V and* ***(C) Pico-Tip*** *for a 1:3 PDMS–OA solution and voltages between 60 and 85 V.*

Nano-Tip A

Two dilutions of PDMS in octyl acetate where tested (PDMS:OA = 1:3 and 1:4 v/v). Both could be dispensed; droplets were somewhat distorted (**Fig. 1A**).

Droplet volume: At a 1:4 ratio of PDMS:OA, the dispensed volume for the 1:4 PDMS–OA solution was 200–420 pl (Fig. 2A, **green line**). Shorter actuation pulses were used for the 1:3 solution and resulted in droplet volumes between 130 and 300 pl (not shown).

Nano-Plotter

Ink-jet Printing of PDMS

Nano-Tip HV

The Nano-Tip HV is designed to dispense liquids of higher viscosity as compared to the other tips. Two PDMS/octyl acetate mixtures were tested (1:2 and 1:3 v/v); both were dosable, with droplet shapes similar to those produced by the Nano-Tip A (**Fig. 1B**).

Droplet volume: For the 1:3 PDMS–OA solution, droplets of approx. 200–700 pl were generated at voltages between 40 and 100 V (Fig. 2A). The more viscous PDMS ink (PDMS:OA 1:2) showed a smaller range of droplet volumes, approx. 300–600 pl (not shown).

Spot diameter: Fig. 2B shows spot diameters for dispensed volumes (**red line**) and **Fig. 3A** photographs of the spots.

Pico-Tip

Due to the different design of the Pico-Tip (smaller orifice), the viscosity of the ink has a higher effect on dispensing. Therefore a higher PDMS dilution (PDMS:OA 1:5 v/v) had to be used. Droplet shapes appeared to be normal (**Fig. 1C**).

Droplet volume: The 1:5 PDMS–OA solution resulted in droplets of about 65–95 pl at voltages between 60 and 85 V (Fig. 2A).

Spot diameter: Again arrays were printed onto silane-modified glass slides, see Figs. 2B (**blue line**) and **3B**.

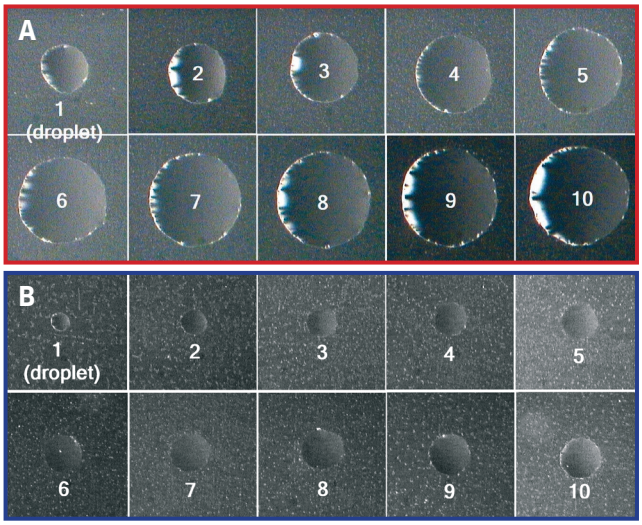

Fig. 3: *Dried/hardened spots of PDMS ink on hydrophobic glass slides for different dispensed volumes (droplets per spot). **(A) Nano-Tip HV**, PDMS:OA 1:3; **(B) Pico-Tip**, PDMS:OA 1:5.*

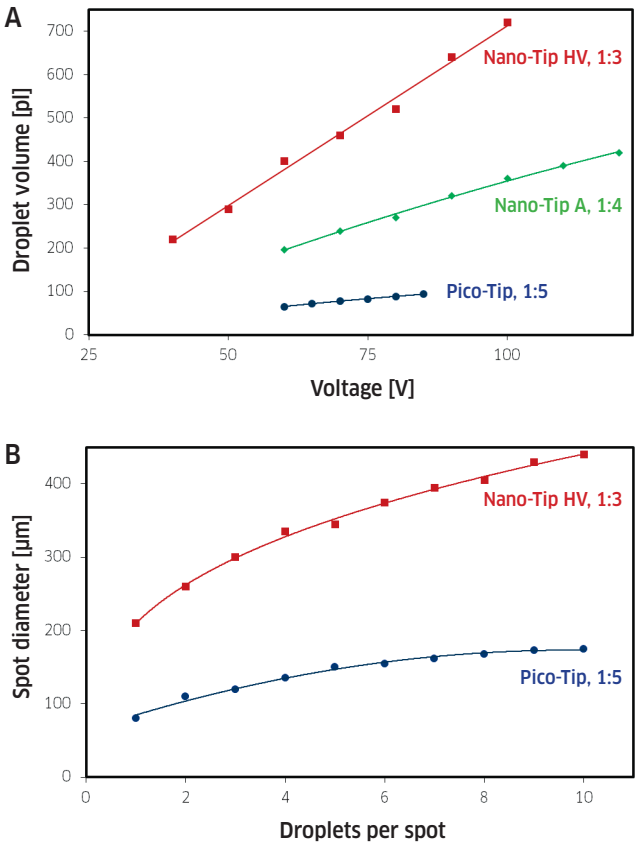

Fig. 2: *(A) Droplet volumes for GesiM piezo tips at different actuation voltages; (B) spot diameters of PDMS inks (estimated droplet sizes: **Nano-Tip HV 500 pl**, **Pico-Tip 90 pl**) on hydrophobic glass slides for different dispensed volumes (corresponding to droplets per spot). The ratio PDMS to octyl acetate (v/v) is indicated.*

Reference

Mikkonen, R., Puistola, P., Jönkkäri, I., Mäntysalo, M.: Inkjet printable polydimethylsiloxane for all-inkjet-printed multilayered soft electrical applications. ACS Appl. Mater. Interfaces 2020, 12, 11990–11997

Note

As the same dispensers can be built into GeSiM's 3D bioprinters (BioScaffolder), this information holds also true for this system..

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Nano-Plotter Application Note

Ink-Jet Printing of Polyvinyl Alcohol (PVA) Solutions Using GeSiM Piezo Tips

Introduction

Polyvinyl alcohol (abbreviation: PVA, PVAL or PVOH) is a widely used polymer with low toxicity that can be used for additive manufacturing (3D printing). Other than most vinyl polymers, PVA is not polymerized from its monomeric units, but synthesized by polymerization of vinyl acetate and subsequent hydrolysis of the ester bonds. Therefore it contains small amounts of polyvinyl acetate, but it is this polymer that helps to make PVA soluble in water. PVA makes excellent transparent foils and films, is adhesive and resistant to oil and organic solvents.

GeSiM ink-jet printing technology can be used for additive manufacturing, as it allows the deposition of small amounts of material at specified positions on a substrate. Dispensing of many layers eventually results in complex 3D structures.

Whether piezoelectric dispensers can print polymers depends on properties of the material, such as viscosity and surface tension. The permissible viscosity of piezo dispensers is often limited to a few tens of mPa·s or less, depending on the dispenser type. Heating would reduce sample viscosity, but is often undesired. As PVA is water-soluble, its viscosity can be lowered by dilution with water, allowing it to be printed at room temperature.

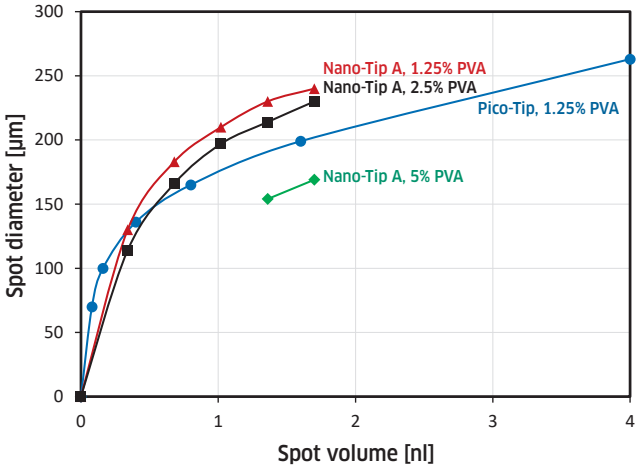

Fig. 2: *Spot diameters for different amounts of droplets per spot, with droplet volumes of 80 pl for Pico-Tip and 340 pl for Nano-Tip A. Smaller numbers of droplets could not be spotted for 5% PVA.*

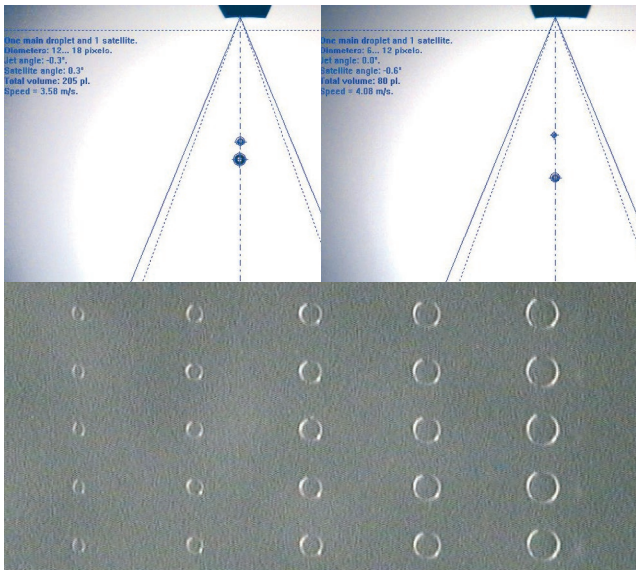

Fig. 1: *Stroboscope pictures of a Nano-Tip A dispensing 5 wt% PVA and a Pico-Tip dispensing 1.25% PVA (top) and a microarray of 1, 2, 5, 10 and 20 PVA droplets (5 wt%) per spot produced by a Nano-Tip A (bottom). The largest spots have a diameter of 275 µm.*

Materials and methods

Water was added to 6.5 g of PVA until a concentration of 10 wt% (weight per cent) was reached and the mixture placed on a magnetic stirrer until it was dissolved. It is difficult to handle with a semi-automatic pipette, as it easily generates bubbles. Solutions of different concentrations (weight percent, wt%) were prepared from this stock solution by dilution with water.

GeSiM's Nano-Plotter NP2.1/E was equipped with different piezoelectric pipettes:

- Nano-Tip A: orifice 50 µm; typical droplet volume 250 pl
- Pico-Tip: orifice 25 µm; typical droplet volume 60 pl

The Nano-Tip A is a piezo pipette with usually very stable dispensing properties over a wide range of fluids and piezo parameters, at a useful droplet volume. The Pico-Tip produces smaller droplets, but its range of permissible solutions (especially with respect to viscosity) and piezo parameters (especially voltage) is substantially smaller.

Nano-Plotter
Ink-jet Printing of PVA

Tips were connected to a PTFE tubing system containing a syringe pump with 3-way valve (diluter). PVA solutions were aspirated from a micro-well plate; an air gap was not necessary due to the water-miscibility of PVA. Droplet volumes were measured with the GeSiM flow sensor while dispensing was controlled in a strobo-scope; the pipette was connected with the pressure compensation vessel during dispensing.

PVA arrays with 1 and 100 droplets per spot were spotted using rectangular voltage pulses (with pre-pulses) onto standard 1×3 inch (26×76 mm²) glass slides (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany), whose surface had been modi-fied by exposure to silane vapour. The contact angle of water after this treatment is around 90°. PVA spots were dried in an oven and the resulting spot diameters measured in a calibrated microscope. Spotting onto polystyrene slides gave comparable results (not shown).

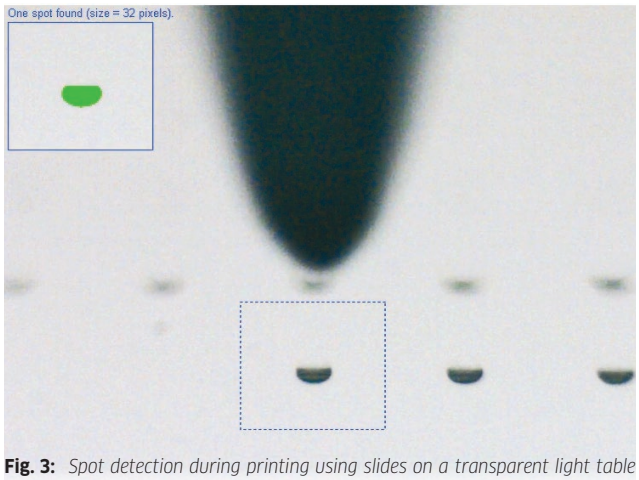

Fig. 3: *Spot detection during printing using slides on a transparent light table and a head camera showing a Nano-Tip A dispensing 4 droplets of 5 wt% PVA per each spot. Dotted lines depict the area of interest.*

Experimental results

10 wt% PVA

This solution could not be dispensed by any means.

5 wt% PVA

Cannot be dispensed by a Pico-Tip, but dispensing at high voltage was possible with a Nano-Tip A. In the stroboscope there is a short phase of a distorted droplet before a regular droplet pattern appears (velocity around 2 m/s).

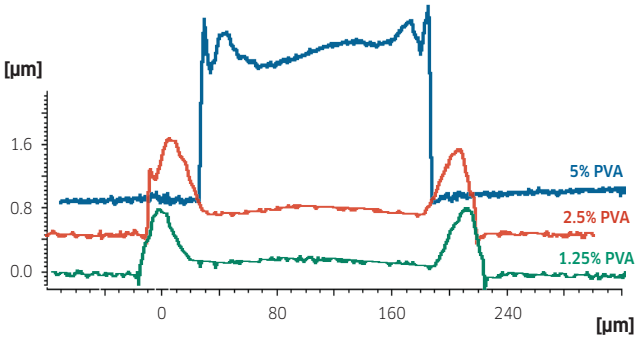

Fig. 4: *Spot height profiles measured with an optical laser method. Five drops each of a PVA solution were dispensed by a Nano-Tip A. The profiles were superimposed and aligned in one diagram.*

Notably, the dosage of only one or two droplets per spot at this high PVA concentration occasionally led to spotting failure, presumably due to drying. Dispensing of more drops per spot, however, was possible.

2.5 wt% PVA

Could not be dispensed with a Pico-Tip, but spotting was possible with a Nano-Tip A (velocity ca. 4.4 m/s).

1.25 wt% PVA

This and more diluted solutions could be dispensed with both Nano-Tip A and Pico-Tip (velocity around 5 / 3.8 m/s).

Spot appearance

Lower concentrations caused ‘coffee ring’ drying artefacts. Higher concentrations gave more uniform spots, apparently due to slower drying (Fig. 4).

In conclusion, we show here that aqueous solutions of polyvinyl alcohol can be dispensed, after some optimization, at concentra-tions ≤ 5% with a Nano-Tip A and ≤ 1.25% with a Pico-Tip.

Note

As the same dispenser types can be used in GeSiM’s 3D bio-printers (‘BioScaffolder’), this information holds also true for this system..

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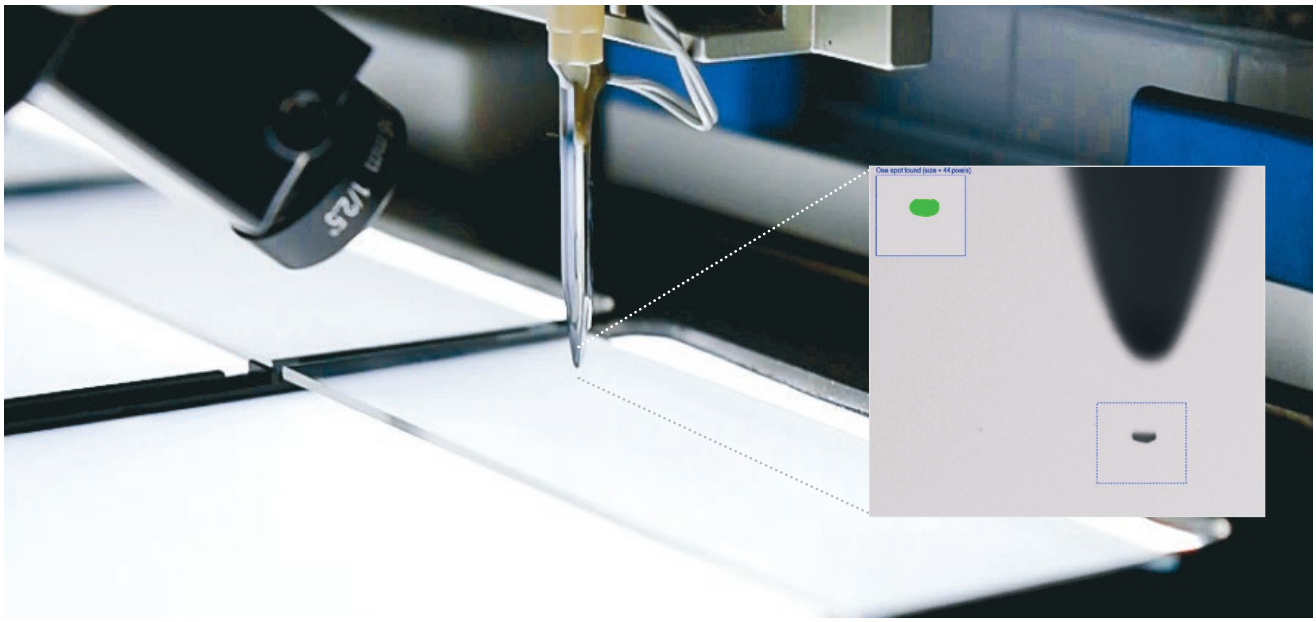
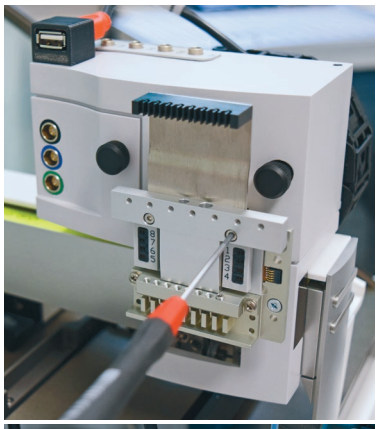
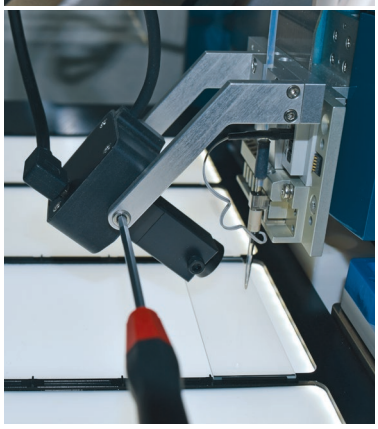
Nano-Plotter Application Note

On-line Spot Control – Live Imaging for Error Correction







For individual spot correction, it is ideal to have a camera observing in real-time whether a spot is being dispensed (before drying) and meets the specifications. This is described here.

Prerequisites

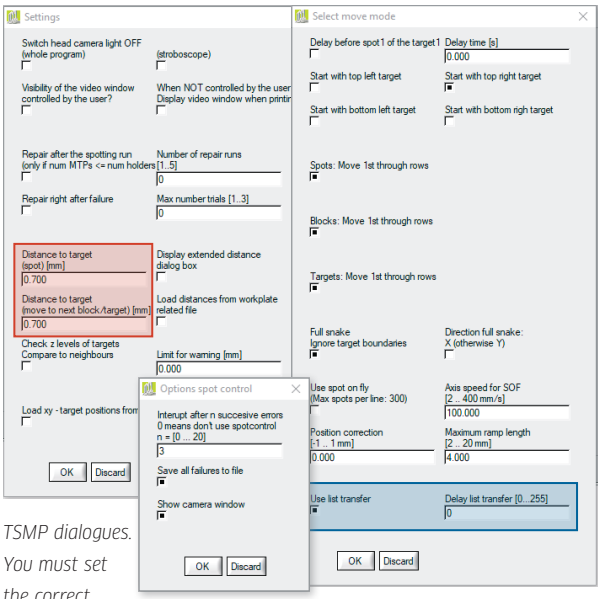
- Only one pipetting channel can be used, with extra-thin tip
- **Cable chain** for the camera cable and camera mount (the Nano-Plotter must be rebuilt)
- **Digital head camera** to visualize the pipette tip, connected via a **USB3** port
- NPC16 v. 2.16.x and *TransferSimMultiPlates* v. 08C14e and accessory programmes
- A back-lit light table for NP2.1 and NP2.1/E and transparent objects (can be customised)

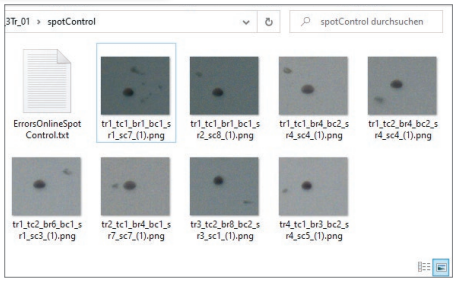
Preparation of head camera and image processing

- Easily mount the head camera and connect it to the USB socket on the pipette head.
- Switch the light table on and start NPC16. Move the tip to a bright spot on the light table (arrow/PgUp/PgDn keys).
- Untighten the screws that hold the camera (see left).
- Choose 'System → Setup → Spot Control…' and swivel the camera until the tip lies right above the square in the control window. Tighten the screws again.
- **Focus:** Define a work plate with source MTP. Position the target group so that the slides ('Rows/Columns' format) align with the light table. Measure all Z-heights.
- Wash/dry the pipette and check for stable droplet formation in the stroboscope.
- In Run mode, press the R button and start *SpotControl_AdjustCameraFocus*.
- Select target group, pipette position for the test (spot row/column, block row/column) and number of dispensed droplets. A spotting distance of ≈ 0.7 mm ensures that the tip does not hide the droplet.

Nano-Plotter
On-Line Spot Control

- Loosen the lens fixation screws and click [OK]. The pipette moves to the chosen position (CAUTION). Manually trigger droplet dispensing (Dispense now? [Yes]) while turning the lens. Repeat until the droplet is focused. Break off with [No] and tighten the screws again.
- **Imaging options:** In Run mode, start *SpotControl_DispenseList_1Spot*. Select target, spot/block position, number of droplets and distance.
- Click [OK]. The tip moves to the position and a burst of droplets is released.
- Using the pre-set image processing parameters, the software tries to find the droplet in the region of interest (ROI, dotted line). Valid pixels are painted green.
- If you do not like the result, go back to the interactive mode and select 'System →Setup → Spot Control…'.
- Now modify parameters, e.g. 'Black /White' threshold, 'Reduce noise' or resize the 'ROI'. You can also enter the [Device Settings].





TSMP dialogues. You must set the correct distance of the tip to the target and 'fast list transfer'. On the right: files in the spotControl folder showing artefacts.

Spotting using on-line spot control

Switch to the Run mode and start *TransferSimMultiPlates* (TSMP). In the first dialogue, activate "Show 'spot control' options" and other important set-up windows.

Select the spotting distance as above. In the 'move mode' window, 'Use list transfer' (extra-fast mode) must be checked. Then set the options for on-line spot control:

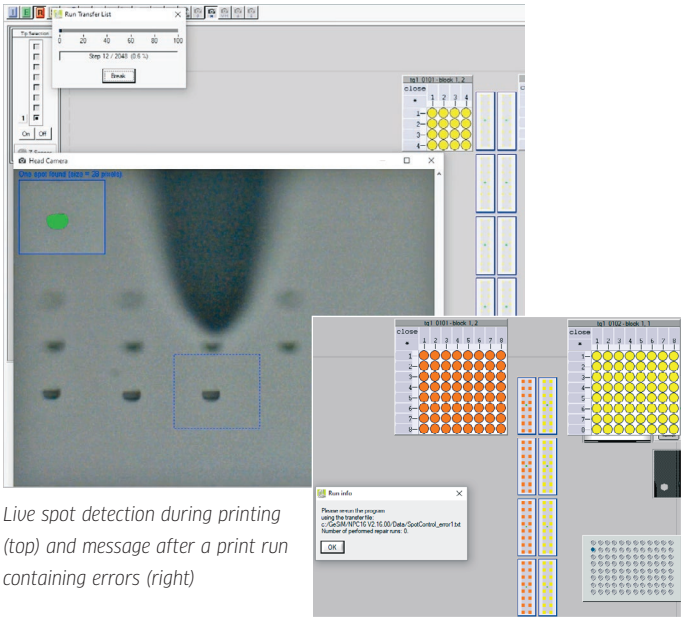
- Number of consecutive errors before spotting is interrupted.
- Error images can be saved.
- The camera can be displayed during spotting.

All other settings are as usual, and you must also load a 'transfer file' that assigns spots to sample wells. After washing, sample uptake and stroboscope test (with optional spot speed measurement), printing starts. A post-spotting stroboscope test will also be performed.

When the number of successive detection errors (e.g. caused by artefacts on the surface, see picture) has reached the pre-set limit, printing will be interrupted to minimise false negatives. Empty spots will be earmarked red, and repair information for them will be added to the repair (error) file. (Samples that fail the stroboscope test will not be printed anyway.).

If errors occurred, you will be told to re-run TSMP for repair.

A *spotControl* folder can be created in the standard 'Report' folder. It contains a text file listing all errors and images of faulty spots to help you find causes of errors.



Live spot detection during printing (top) and message after a print run containing errors (right)

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