# An efficient manufacturing process for highly complex biochips using laser printing technique

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## Abstract

*At present laser printing has not been drawn many attention as a manufacturing technique. But it offers an interesting alternative to inkjet printing for many digital manufacturing applications. We first give a short comparison of both printing techniques before presenting an efficient production method for highly complex biochips – peptide arrays – based on laser printing. Peptide arrays are powerful tools for developing new medical substances as well as for diagnosis and therapy techniques. The new production method will enable the potential of peptide arrays to be effectively utilized for the first time.*

## Introduction

Different from inkjet printing the laser printing technique has not been recognized as a powerful tool in manufacturing yet. But due to its operation principle it has many advantages compared to the meanwhile widely applied inkjet printing. A sketch of the operation principle of a laser printer is shown in fig. 1.

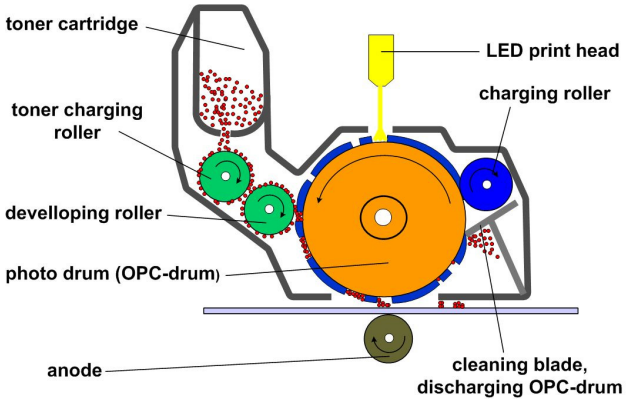


***Fig. 1:** Operational principle of a single-pass laser printer for mono-component toner (system Oki Company)*

Digital printing is used in manufacturing for the mask less application of highly resolved coatings. The substances to be deposited are stabilized in a jetable ink or in the case of laser printing they are embedded into a toner powder which has a defined electrostatic charging behavior and size distribution. Characteristic properties that qualify a toner be suitable to laser printing are the size distribution of the particles and their charge-to-mass ratio, q/m. The electrostatic charge of the toner results from triboelectric charging of the particles against the developing roller (fig. 1) or – in the case of a two component toner – against the carrier particles. Triboelectric charging is based on the difference of the emission energy of electrons (the work function) of two materials in contact and the electron tunneling probability as a function of gap distance between the two surfaces [1]. Therefore triboelectric charging requires an intimate contact of the surfaces which is provided by stirring the toner – carrier mixture or rubbing the toner particles between two rollers (the toner charging and the developing roller in fig. 1 which rub against each other).

In xerographic processes the toner transfer is controlled by the balance of the electrostatic and adhesion forces. The adhesion forces dominate; in average they are roughly 2–10 times larger than the electrostatic forces. The adhesion force-to-mass ration $F_{ad}/m$ scales with the particles radius as $1/r^2$ or $1/r^3$ (there is some uncertainty depending on the model of adhesion used for toner particles [1]) whereas q/m scales with 1/r. Without concern which relation exactly holds, the size distribution of the toner particles (and their surface coating) strongly influences the toner transfer onto the opc-drum and from the opc-drum onto the probe carrier.

Some advantages of inkjet and laser printing are summarized in the following table. Depending on the specific application the one or the other advantage may be of importance:

**Inkjet printing (drop-on-demand)**

+ Printing onto structured and shaped surfaces.
+ No damaging of the surface by contact printing
+ Broad variety of liquids can be jetted despite the range of viscosity for inks is small
+/- High printing resolution possible, but at cost of the reliability of the printing process

**Laser printing (LED-printing)**

+ Xerography is a very stable and reliable process – easy maintenance, no clogging of nozzles
+ Suppression of uncontrolled chemical reactions because chemical and biochemical reactants are encapsulated in the toner powder during printing – no interchange reactions are possible as it easily happens by the intermingling of drops
+ High printing resolution
+ In the case of solid substances a much higher fraction of solids can be embedded into a toner than into a jetable suspension.

Due to their different properties both printing techniques complement each other not only for graphical but as well for manufacturing applications. A convincing production method enabled by laser printing is the efficient manufacturing of highly complex biochips – peptide arrays. We first turn to the importance of peptide arrays for biochemical research and medical applications before we present the manufacturing process.

## Biochips

Biochips promise to advance biotechnology and medicine by providing the opportunity of massive parallel screening for (chemical) binding partners. They are used for the development of new medical agents, vaccines, or methods for fast diagnosis of diseases. In these screenings libraries of proteins or peptides (short protein fragments) are probed with molecules of interest as for example important proteins in pathogens, cancer cells or viruses in order to identify binders of diagnostic or therapeutic capability.

Proteins consist of 20 amino acids which are linked to long chains. A typical protein has a length of about 500 amino acids. However, protein fragments, so called peptides, with a length of 12 to 20 amino acids are sufficient for identifying characteristic binding reactions. These screenings can be regarded as search for a biological key fitting to a lock. But the problem of this task is the enormous number of peptides needed.

About 100.000 peptides are necessary in order to represent each of the approximately 1000 proteins in a bacterium – in the form of 100 overlapping peptides – and a staggering 500.000 for a malaria pathogen. On today peptide arrays are produced by a spotting technique that uses a robot to dab individual amino acids onto a paper-like membrane. A maximum of 10.000 peptides fit onto a probe carrier where the main drawback is their price: An individual peptide spot costs around 5€, adding up to almost 50.000€ for a full array – too much for most promising applications.

The market of biochips is predicted to grow fast where the emphasis lies on basic research, drug discovery, and molecular diagnosis [2]. But the spreading of this technology is impeded by the high production costs of biochips. Meanwhile lithographic methods allow for the combinatorial synthesis of highly dense oligonucleotide chips (DNA- chips), but the more important peptide arrays – which can not be produced by this elegant method – lag behind. But laser printing of peptides could be the way out.

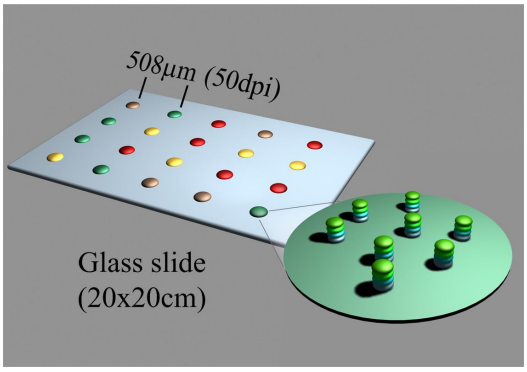


***Fig. 2:*** *Layer by layer manufacturing of a peptide array on a glass slide*

## Laser printed peptide arrays

The idea is to synthesize peptides layer by layer from amino acids on a glass slide where the laser printer is used as a highly efficient micro dispensing tool. The process works as follows: Protected amino acids (instead of colors) are embedded into toners that have similar physical properties as commercial color toners. Since peptides and proteins consist of 20 amino acids, an according number of amino acid toners and printing units in the printer are needed. In the first run spots of the 20 toners are printed in an array pattern onto a coated glass slide where the single spots are well separated from each other, see fig. 2. Then the slide is removed from the printer and heated up to about 90°C. The toner particles melt, turning the toner matrix into a solvent. At this step the embedded amino acids are activated and couple to the coating of the carrier. Subsequently the toner matrix and the charge control agents are washed away and the coupled amino acids are deprotected. Now a single layer of the amino acids is bound on the glass slide. The complete synthesis cycle is depicted in fig. 3.

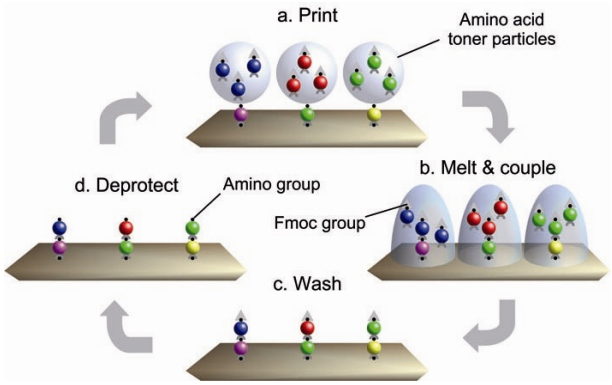


***Fig. 3:*** *The synthesis cycle includes printing of the amino acid toners (a), coupling of the amino acids by melting (b), washing (c), and deprotecting steps (d).*

During printing, the amino acids are processed in a dry state preventing any undesired chemical reactions. The coupling reactions are started in a controlled manner by heating the carrier outside the printer. After completion of the first synthesis cycle the probe carrier is put into the printer again and a second layer of amino acid toner is printed exactly on top of the first (fig. 2). After the next synthesis cycle amino acid chains of length two are generated on the glass slide. When repeating this printing and synthesis cycle 12–20 times over 155.000 peptides of the corresponding length are parallel synthesized on a glass slide of 20 by 20 cm.

### *Benefits*

Compared to the state of art laser printed peptides arrays are much more complex, i.e. contain much more peptides. At present a glass slide measuring 20 by 20 cm contains over 155.000 micro spots, and can be manufactured much faster at a price that is at least *100 times* lower than that of conventionally produced peptide arrays. The arrays can now be offered for a few cents per peptide. Current developments will increase the number of micro spots to over 560.000 on the same area and consequently further reduce the price. This new production technique will advance research in life sciences by enabling many important projects which are not possi-

ble on today due to the lack of highly complex and affordable peptide arrays.

***Fig. 4:*** *Prototype peptide laser printer*

## Manufacturing technology

### *Printing technology*

Conventional color laser printer print four color toners onto paper or transparency; the peptide laser printer prints twenty amino acid toners onto glass. The first prototype is shown in fig. 4. For each of the 20 amino acids a printing unit with a 1200dpi LED-array (Oki Company) is provided, where the printing units are arranged in a line, see fig. 5. The glass slide is mounted on a linear slide which moves below the opc-drums. The linear motion is synchronized with the rotation of the opc-drums and the toner is transferred directly from the opc-drums to the glass slide (single-pass printing technique).

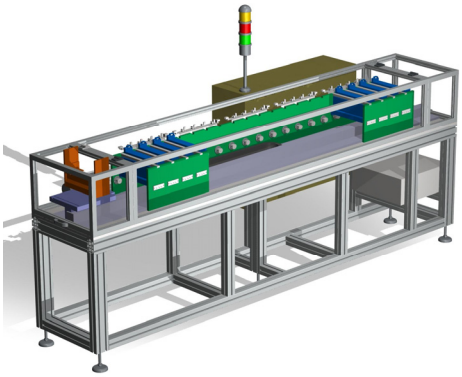

***Fig. 5:*** *Construction of the prototype peptide laser printer*

Toner transfer onto glass is more difficult than onto paper or transparency due to the stiffness of the material and its smooth surface. Since the adhesion forces of the toner particles are typically larger than the electrostatic forces a conformal (local) contact of the probe carrier to the opc-drum is required. The uniform local pressure of the glass slides against the opc-drums and the precise positioning of the probe carrier are achieved by the mounting. The single-pass printing technique implemented here works for glass slides being not too thick, i.e. which are flexible within the range of about 0.2mm. A transfer anode which supports the toner transfer by an electrostatic field (about 4kV/mm) is integrated below the glass slide.

According to the principle of single-pass printing 19 opc drums move over the toner spots deposited by the first printing unit. Considering a finite toner transfer rate, each drum is expected to remove a fraction of the already deposited toner. From this viewpoint it is not clear if single-pass printing with four color toners onto paper also works with 20 toners onto glass. But different from commercial color laser printers the toner spots deposited by different printing units in a single run do not overlap (fig. 2). Therefore the regions of the opc-drums which contact the already deposited toner are still fully charged; the opc-drums are exposed at separated spots only. This property reduces the rate of removed toner enabling single-pass printing with 20 toners onto glass.

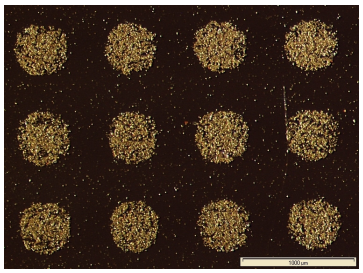

***Fig. 6:*** *Magnified amino acid toner spots*

The accuracy requirements of the images are much higher than that of commercial printers. To produce a peptide array 12 – 20 printing layers each with 20 different amino acid toners must be printed exactly on top of the previously generated spot pattern. After each run the probe carrier is removed for chemical coupling-, washing- and deprotection steps and put again into the printer. The arrays printed by the first prototype contain ~400 spots/cm$^2$; this corresponds to a point to point distance of 508µm, see fig. 6. Its successor will manage to print arrays with half the point to point distance yielding four times as many peptides per area. An experimental rig for the further development of this printing technology is shown in fig. 7.

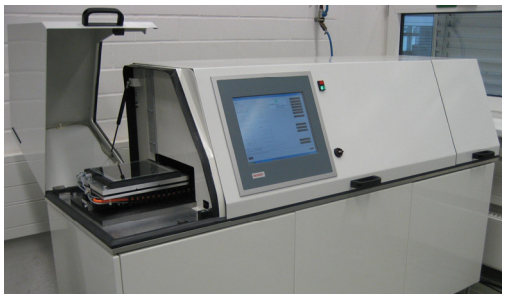

***Fig. 7:*** *Laser (LED-) printer for printing onto glass slides. The probe carrier and mounting are shown.*

### *Amino acid toners*

Essential for the success of the peptide printing method is the development of the 20 amino acid toners. The amino acids embedded into the solid matrix are protected against uncontrolled chain

reactions (fmoc-chemistry), but activated to enable the coupling reaction with the derivatisized surface (coating) of the glass slide. To assure a defined triboelectric charging of the toner particles charge control agents and stabilizers are added to the matrix material. These ingredients need to be compatible to the chemistry of the synthesis cycle (fig. 3) which excludes many substances. Compared to color toners the melting point of the toner matrix must be lower (< 90°C) to prevent undesired side reactions during peptide synthesis.

To obtain a low melting point of the toner matrix and to optimize the coupling reactions solid solvents are used which are higher homologues of standard (liquid) solvents traditionally used for peptide synthesis, e.g. DMF(l) vs. DPF(s). Coupling yields within this solid-liquid environment proved to be comparable to standard liquid processes.

Interestingly enough, the highly sensitive amino acids prove to be surprising stable when encapsulated into toner particles. The decay rate of the most sensitive amino acid toner is about 5% per months (stored at 25°C) while the corresponding amino acid ester (Fmoc-Arg-OPfp-ester) decays in a conventional solution within minutes. This is an enormous advantage for this manufacturing process for peptide arrays.

The physical properties (q/m, size distribution) of the amino acid toners are very close to those of commercial color toners. As a result the printing resolution and the toner transfer rate are comparable. In fact, at the beginning of the project the physical properties of commercial color toners (from Oki Company) were defined as the interface for the parallel development of the peptide laser printer and the amino acid toners.

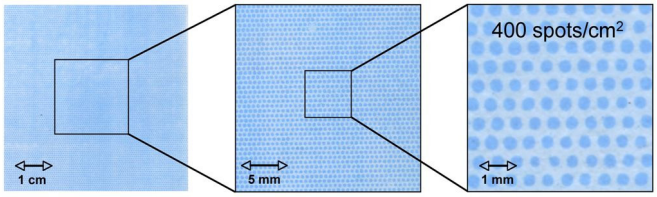


***Fig. 8:*** *Printed and coupled amino acid on a glass slide (stained)*

## Biochemical functionality of peptide arrays

To test the biochemical functionality of the resulting arrays 5,500 different peptides were synthesized on microscope slides (400 peptides per $cm^2$) that display permutated Flag- and Myc-epitopes, see fig. 9. When the arrays were incubated with the corresponding antibodies, all of the permutated peptide epitopes showed the expected staining pattern, which correlates in relative intensity with previously published results [3, 4].

A description of the comprehensive chemistry and biochemistry of the combinatorial synthesis of peptide arrays is beyond the scope of this paper. It can be found in [5].

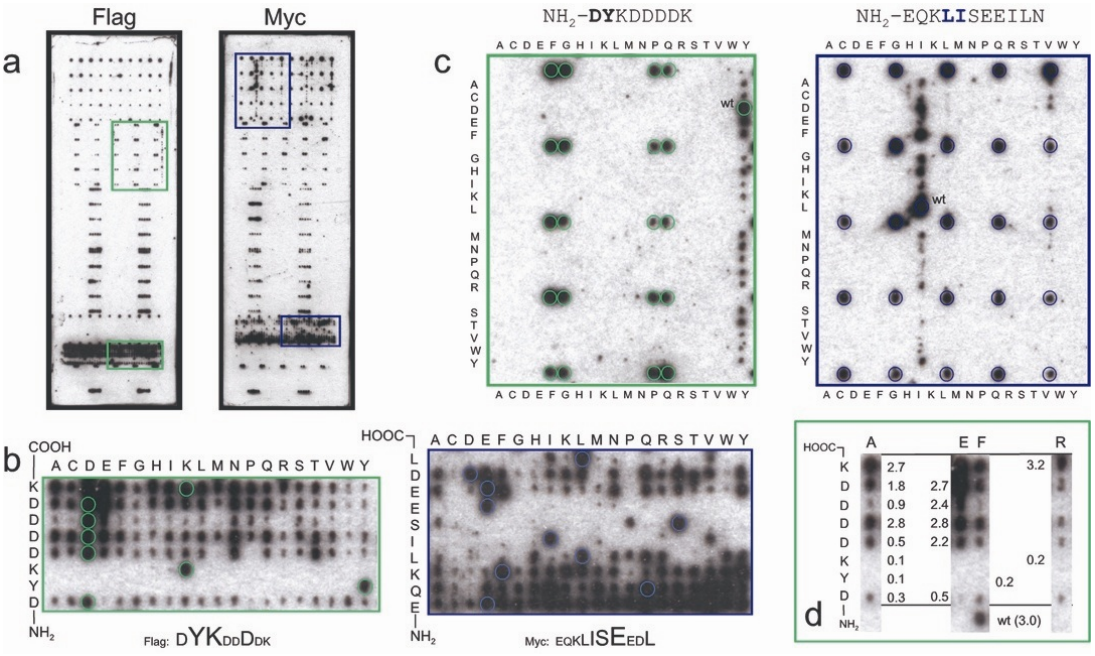


***Fig. 9:*** *Combinatorial synthesis and immune staining of a peptide array. (a) A glass slide with ~5,500 peptides generated by combinatorial synthesis is stained with Flag or Myc antibodies. Colored frames delineate the enlargements shown below. (b) Epitope runs for Flag and Myc epitopes. Every amino acid position of each epitope was exchanged for all 20 different amino acids to characterize the binding requirements of epitope specific antibodies. The epitope specificity deduced from individual experiments is given in the sequence written underneath, where the letter size of the amino acid symbols reflects their importance for antibody binding. (c) Epitopes permutated at two positions that are highlighted by color in the peptide sequence. Interspersed wild type sequences are encircled. (d) Staining intensity of variant Flag epitopes from the epitope run in (b).*

## References


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## Author Biography

*Stefan Güttler obtained his diploma in theoretical physics from the University of Freiburg, Germany (1996). He obtained his doctoral degree (1999) at the Max-Planck-Institute for Physics of Complex Systems in Dresden where he worked on signal processing in technical systems. In 2000 he joined the Fraunhofer Institute for Production Engineering and Automation in Stuttgart where he develops digital printing technologies (laser and inkjet printing) for manufacturing applications. Beside applications in biochemistry a focus is on printing of (ceramic) particles. For developing the efficient manufacturing process for highly complex biochips the Science Price of the German Stifterverband 2008 was awarded.*