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Manufacturing & Processing Technologies

Developing low-volume dispensing systems for biosensors and IVDs

Point-of-Care Technologies

Applying piezo-optical methods to next-generation POC devices

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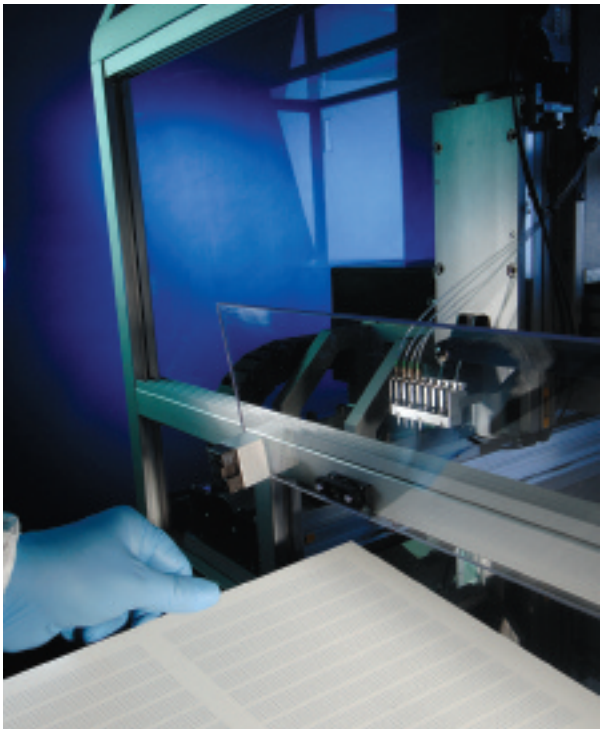
Low-volume dispensing of biomaterials for biosensors and IVD tests

Fabricating biosensor arrays requires reagent-dispensing systems that can produce hundreds of thousands to millions of dispenses with high precision and accuracy. BY ANTHONY V. LEMMO, BYRON DONEEN, THOMAS C. TISONE, AND BARBARA McINTOSH

Following advances in microelectronics, biosensor designs have become increasingly complex and focused on miniaturization. The demand for simultaneous measurements of multiple analytes has also stimulated the development of high-density arrays. As a result of such demand and the advancing capabilities of patterning technologies, recent research and development programs are aimed at developing sensor arrays that contain tens to thousands of individual sensors for devices that are as small as 1 cm^2 in size.



Fabricating such devices requires reagent-dispensing approaches capable of delivering volumes ranging from the low microliters to picoliters. Reducing volumes decreases the cost of expensive reagents, increases surface-dependent reaction rates, and promotes the adoption of multiplexed IVD devices. Such benefits have resulted in applying low-volume dispensing to various research areas including biosensors, biochips, protein arrays, and cell arrays.

The dispensing systems must be compatible with a range of reagent classes, including organic solvents, biological fluids, polymeric solutions, and the traditional combination of buffers and enzymes. The systems must be robust enough to produce hundreds of thousands to millions of dispenses with a high level of precision and accuracy. The systems must also function in a production environment using less-skilled labor and comply with rigorous regulatory requirements.

This article reviews process parameters that affect accuracy, precision, and reproducibility as measured by the coefficient of variation (CV) of drop volume. Reducing variability is the key to minimizing rejected parts and other manufacturing costs, and to gaining acceptance by the medical community and patients. While variability can be minimized at the level of chemical formulation, and through inclusion of appropriate electrode controls and regular recalibration by specialists or patients, reducing the variability of the dispensed volumes remains essential.

Biosensor Definition and History

The simplest definition of a biosensor is a device for detecting analytes that combines a biological component with a physicochemical detector component (see Figure 1).[1] Biosensors are used with printed circuits and surface acoustic wave or other acoustic elements, and incorporate electronics or signal processors (readers) that display the results in a user-friendly way.

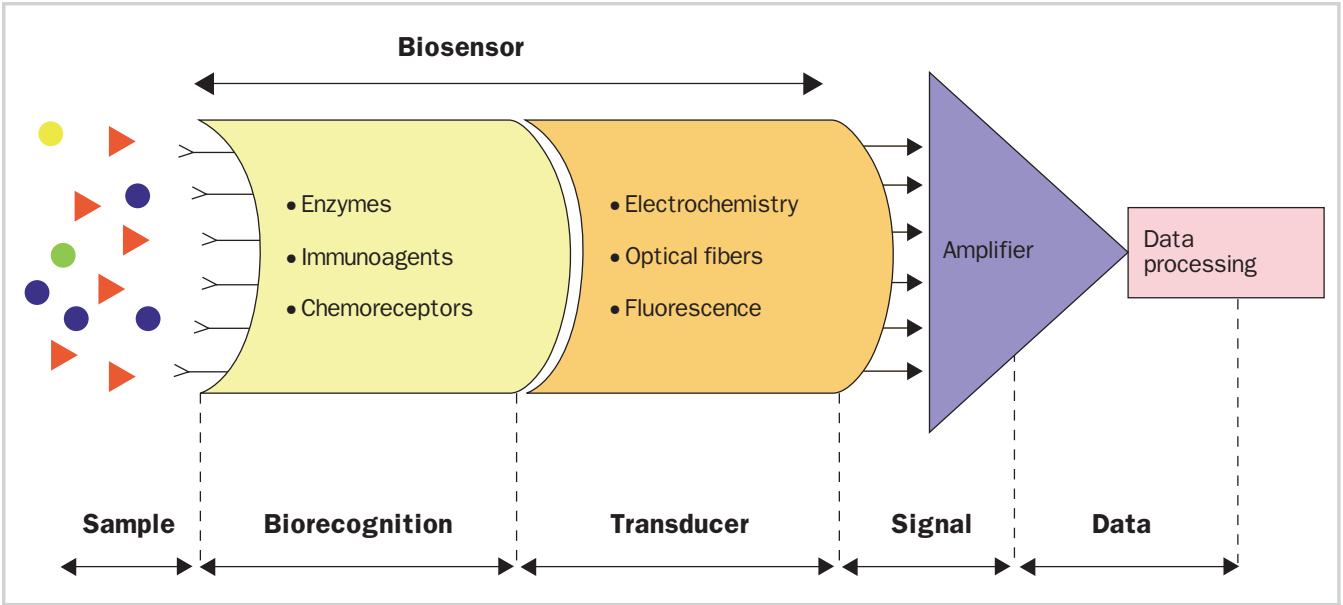


Figure 1. A biosensor process from sample addition through to detection.

Biosensors incorporate a biological sensing element, such as an enzyme, antibody, antigen, nucleic acid, etc., with a physiochemical transducer. When an analyte is presented to the transducer, a chemical reaction occurs and provides an electric signal that is proportional to the concentration of the analyte.

The biosensor process requires a base electrode, and the base material is typically made of plastic. A number of elements can be printed onto the plastic including the following: a carbon/graphite mix, conductors, reference electrodes, and insulators/dielectrics.

The biological sensing element is then applied to the transducer. A printing process (e.g., screen printing) is unsuitable for most biological materials, particularly when high temperatures are used to cure the printed electrodes. An alternative method is to dispense the materials dissolved or suspended in the buffer. Researchers will often need to experiment with a range of variables when developing biosensors, including the following:

- Changing drop volumes while maintaining low CV.
- Adjusting drop chemistries.
- Adjusting drop spacing or pitch.
- Applying multiple analytes.
- Adding polymers-forming networks that favor the accessibility of analytes or filter contaminants.

The first biosensor was developed in 1962. At that time, Leland C. Clark, PhD, demonstrated a biosensor that measured a glucose concentration in a solution by using what is now known as the Clark oxygen electrode. Clark had the ingenious idea of placing an enzyme very close to the surface of a platinum electrode. The enzyme would react with the oxygen and the sugar, and Clark theorized that the enzyme activity could be traced by monitoring changes in oxygen concentration.[1]

The glucose biosensor is still the most prevalent biosensor in the IVD marketplace today. Moreover, the demand for glucose monitoring is expected to increase. Studies have concluded that, "The increasing rate of obesity and the alarming rise in the rate of diabetes in the industrialized world is driving a need for more biosensors to monitor diabetic patients' glucose levels."[2] For example, the estimated diabetic population in the United States is approximately 20 million people, and only 66% of them have been diagnosed and are receiving treatment.[3]

In addition to conventional medical sensors, pharmaceutical research is promoting the development of new rapid assay biosensors to speed up drug discovery. Military and security applications are driving the use of new rapid detection biosensors for biowarfare agents. Biosensor formats have also been used in food safety, environmental monitoring, and hospital and public sanitation. Environmental monitoring includes long-standing applications for wastewater, and industrial gases and particulates.

Biosensor Development and Production

The biosensor format is ideal because it produces analytical data that allow end-users to make informed decisions quickly. The biggest difference with standard clinical laboratory assays is that biosensors require fewer steps from sample addition to quantitative results. In most cases, biosensors need to be small, portable, and quantitative. Biosensors also encompass the advantages of lateral-flow devices because they are constructed of different materials, have different shapes, and are highly precise. The business model that underlies the

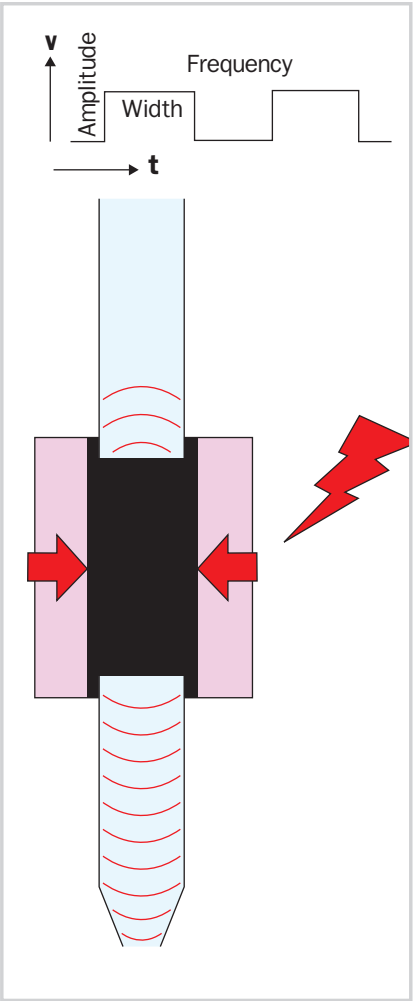


Figure 2. The piezo dispensing technology.

Figure 3. The Scienion s11 piezo dispensing system.

development of biosensors requires low production costs and stable products with long shelf lives, and demonstrates the clinical or environmental needs.

Like most rapid tests, biosensors can be produced in either a batch or in-line mode. Batch mode consists of producing parts in lots or batches, while an in-line mode employs a continuous roll-fed system. After dispensing, such steps as drying, laminating, and vision inspection follow until the biosensors are cut or singulated from the cards. Like dispensing, the cutting can be done in either a batch or in-line mode of production.

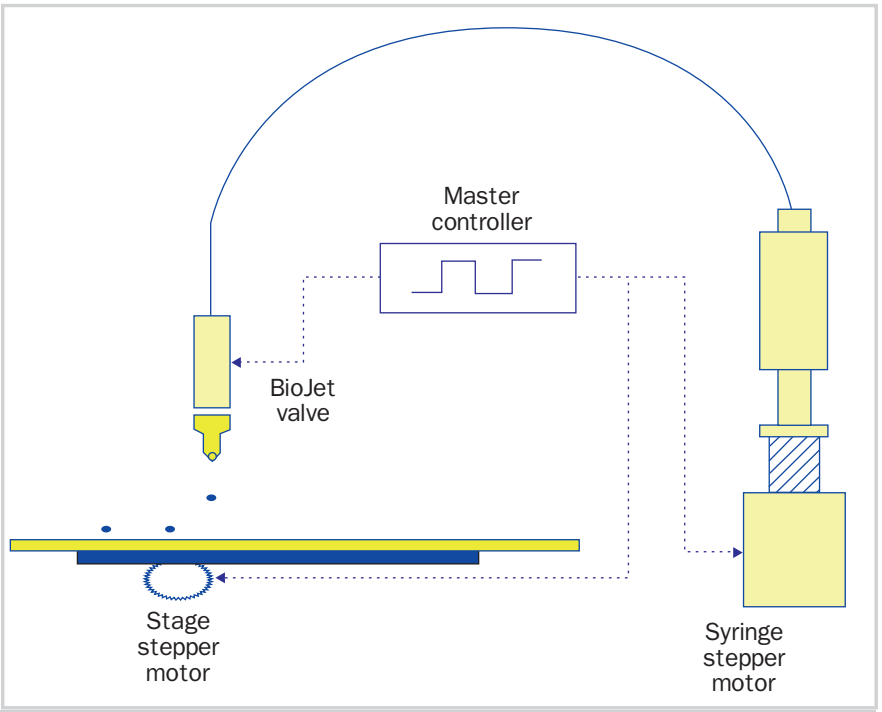


Figure 4. Schematic of the BioJet Plus dispensing technology. The microsolenoid valve is coupled to a high-resolution syringe pump as well as the x-y-z motion.

In biosensor production, there are five key phases. First, the sensor's architecture is designed and constructed. Although several detection modalities exist (e.g., optical, magnetic, piezoelectric, acoustic, and electrochemical), the most common is electrochemical. The second issue is to determine the method of fabrication. Options include microfabrication and screen printing. The biologics come into play in the third phase in which the choices of materials are antibodies, enzymes, nucleic acids, receptors, and cells. Once this is decided, the fourth phase is to determine the method of immobilization. Typical examples are adsorption, entrapment, microencapsulation, and covalent attachment. The fifth and final phase is signal processing.[4]

Since the 1960s, the sensor format has advanced significantly in the materials and formats used, and in the transducing and detection processes. Some of the key challenges in continuing biosensor development include the stability of the bioactive layer, the reproducibility of the biosensors being produced, and the technology needed to work with the small volumes mandated by new formats.

As stated above, research and development programs are aimed at developing sensor arrays that may contain tens to thousands of individual sensors for devices as small as one square centimeter. Fabricating such devices requires reagent-dispensing approaches that can deliver volumes ranging from the low microliters to picoliters and can handle multiple reagents with distinctive properties.

Dispensing Technologies

Several dispensing technologies operate within the submicroliter range. The first step is to choose between a contact and noncontact mode for dispensing reagents. Traditional contact-based pin printing can deposit picoliter volumes or higher depending on the pin type. Although

												Mean
2339	2035	2252	2520	2303	2260	2398	2427	2480	2285	2352	2371	2335.17
2325	2371	2296	2324	2322	2331	2289	2435	2317	2307	2357	2281	2329.58
2676	2307	2256	2336	2179	2325	2369	2278	2300	2277	2306	2326	2327.92
2363	2378	2308	2432	2261	2313	2298	2322	2294	2360	2334	2291	2329.50
2386	2504	2282	2398	2440	2329	2346	2465	2436	2400	2589	2364	2411.58
2266	2290	2333	2205	2399	2296	2321	2403	2322	2381	2459	2322	2333.08
2368	2317	2192	2193	2317	2365	2404	2326	2392	2242	2372	2397	2323.58
2282	2338	2143	2469	2346	2411	2380	2392	2406	2379	2337	2322	2350.42

	Plate	Mean	STD	%CV
		2342.60	86.48	3.69

Table I. Values are relative fluorescence units (RFU) in a 96-well plate into which 100 nl of fluorescein-labeled PBS was dispensed in a single dispense program. Mean activity for each row is shown in the right column. Mean, standard deviation (STD), and percent coefficient of variation (CV) for the entire plate are shown at the bottom. Uniform dispensing is indicated by the small %CV.

using a capillary silicon pin allows better CV than traditional metal pins, silicon still risks damaging the substrate surface due to contact. Another contact method is the FrontLine technology by BioDot Inc. (Irvine, CA), which is less damaging to the substrate surface. The FrontLine technology drags a flexible microtube that holds a meniscus across the substrate. The flexible tubing of the FrontLine system has been used for both reagent and biologic deposition.

Noncontact dispensing options include acoustic, piezo, or ink-jet dispensing. Acoustic droplet dispensing uses a focused acoustic beam to eject a controlled droplet from an open pool of liquid. When the acoustic beam strikes the liquid surface, the pressure causes the fluid to rise with the droplet breaking off. Acoustic dispensing supports extremely low dispense volumes that are less than 1 pl per droplet.

Piezo dispensing yields volumes in the 100 pl to the low nanoliter range by applying a voltage at high frequency to a ceramic or piezo element that has contact with a glass nozzle. The current causes the ceramic to contract, which also causes the glass nozzle to contract. The result is the ejection of individual droplets (see Figure 2). Piezo dispensing is influenced by the applied voltage, pulse width, and frequency. Changes in these parameters will affect the dispensing results. For example, the sciFlex piezo dispensing system by Scienion AG (Berlin) has the piezo crystal mounted near the glass capillary (see Figure 3). This close proximity of the piezo element to the dispense tip results in uniform action and droplet ejection.

Plate	Mean RFU	Plate %CV	Mean nl
1	3896.13	1.95	101.01
2	3878.74	1.97	100.76
3	3853.57	1.87	99.82
4	3901.04	1.86	101.01
5	3891.17	2.12	100.76
6	3888.24	1.84	100.69
7	3833.11	1.98	99.31
8	3873.30	2.02	100.31
Mean	3877.43	1.94	100.48
SD	23.18	0.09	0.61
%CV	0.60	4.77	0.60

Table II. Relative fluorescent units used to calculate plate CVs and dispense volumes.

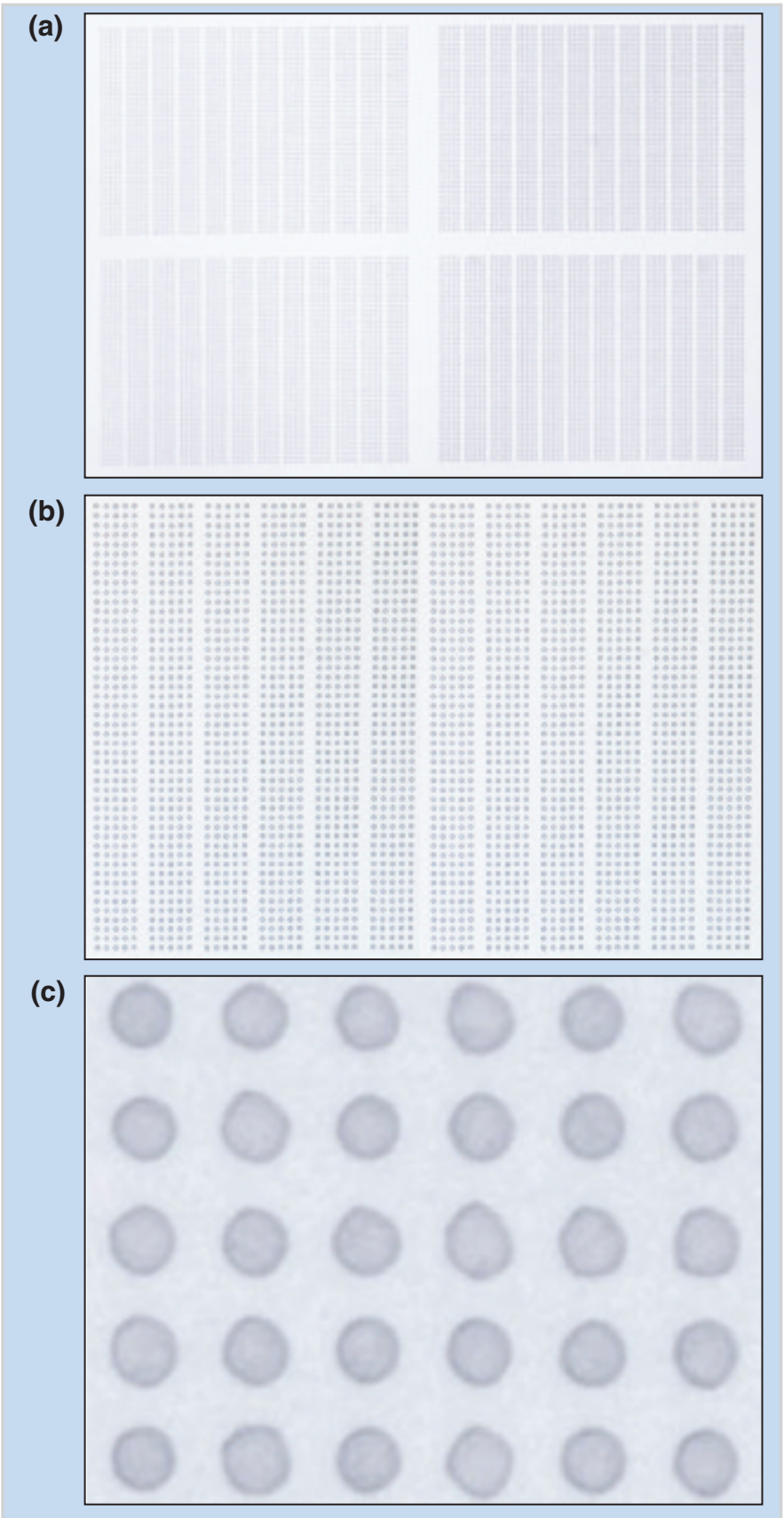


Figure 5. (a) Results of a biosensor sheet printed with the BioJet Plus technology. (b) Closeup photo of the biosensor sheet. (c) Zoomed-in photo of the biosensor sheet highlighting the spot morphology and alignment.

Ink-jet printing or spotting involves the use of a pressure source (hydraulic or pneumatic) and a microsolenoid valve as a drop former. The valve is programmed to open and close under pressure from the pressure source, causing drops to be ejected. For example, the BioJet Plus by BioDot is a quantitative, modified ink-jet mechanism that prints droplets in the nanoliter to low microliter range. The BioJet Plus technology combines a high-resolution syringe pump as the hydraulic pressure source with an actuating microsolenoid valve for dispensing, and is coupled to x-y motion for synchronized dispensing (see Figure 4).[5–8]

The BioJet Plus technology can also process many reagents, encompassing a range of fluid properties. For biosensor production, such reagents include buffers, antibodies, enzymes, and cells. In some novel sensors, biologics are coupled to beads, glass, or coatings of polymers acting as protectants or filtration surfaces.

The BioJet Plus can vary the drop volume from 10 nl to 4 µl as a single dispense. Due to its noncontact nature, it can dispense to high-density plates (e.g., 3456-microwell plates with a well pitch of 1.125 mm) or proprietary biosensor formats that have targeted microwells. The BioJet Plus can also deposit reagents in either aspirate/dispense or continuous-dispense mode. This option depends on the application and the scale of production. Typical CV for the BioJet Plus at 20 nl is less than 10%; as the dispense volume increases, the CV decreases to approximately 4% or less (see Tables I and II).

Volume and Positional Accuracy

Robust dispensing of fluids at low volumes is a technically demanding process that requires rigid control of multiple variables. The primary factors and system components contributing to accurate and repro-

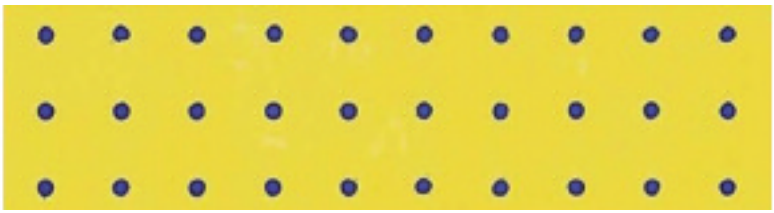


Figure 6. An array that was deposited to the water-sensitive slides.

Figure 7. The AD3400 system configured with BioJet Plus dispensing and online vision systems for automatic batch processing.

Figure 8. An in-line reel-to-reel system that offers continuous processing.

ducible low-volume dispenses are the following: the dispenser, the environment, the fluids in the system, the target substrate, programming design, process design, and dispenser cleaning and maintenance.

Degassing the fluids is a critical element for ensuring consistent, high-quality dispensing. In a hydraulically driven system, the cycling between pressurized and depressurized (valve opening) states can continuously generate bubbles. While off-line degassing is helpful, it does not prevent bubbles from reforming.[9]

To maintain the required steady-state pressure that the BioJet technology requires, BioDot has integrated a method to achieve efficient degassing in a flow-thru vacuum chamber, or In Line Degasser. This chamber contains a single amorphous perfluorinated copolymer (Teflon AF) degassing membrane. The gas-permeable membrane is acted on by a continuously vented minivacuum pump with a unitary PTFE diaphragm. This degassing method reduces the dissolved oxygen of water from 7–8 ppm in a fully aerated solution to 3–4 ppm after passing through the degassing chamber. Several vacuum degassing modules may be mounted in parallel on one system. This degassing technique also eliminates the time required for degassing off-line prior to any dispensing experiment.

Table I shows the CV data based on the relative fluorescence units (RFU) of 96 dispenses of 100 nl of sodium fluorescein. Table II and Figure 5 show the results from a production run of 288,000 dispenses. Using ten 30 × 40-cm sheets, this production run dispensed on each sheet 28,800 drops of blue dye in phosphate-buffered saline (100 nl each). Figure 5a shows 28,800 dispenses on one sheet; Figure 5b is a close-up view of one quadrant of a sheet; and Figure 5c is a further close-up view that shows the details of one column of dispenses to highlight spot uniformity.

After dispensing the 28,800 spots on each sheet, the drop-to-drop CV and average volumes were measured based on RFUs (see Table II). After dispensing 288,000 spots, the overall CV is low (1.94%), and it changed only slightly from the start to the finish of the production run. The card-to-card variability was also statistically insignificant as indicated by the analysis of variance (P = 0.01) of the plate CV. In addition, the mean measured values for volume closely match the target 100-nl volume. A confirmation of accuracy was derived from a comparison with a fluorescein standard curve.

Besides precise and accurate volumes, biosensor production requires high positional precision. To demonstrate the positional precision and accuracy of the BioJet Plus technology, an array of 50 spots produced by a series of 20-nl dispenses was analyzed (see Figure 6). To generate this image, 20 nl of deionized water were dispensed on a water-sensitive substrate. The untreated yellow slide turns blue when the fluid contacts the substrate. This substrate was scanned at 600 dots per inch on a flatbed scanner, and the resulting image was analyzed with ImageJ software by the National Institutes of Health (Bethesda, MD).

An analysis of the spot center of mass (both x and y coordinates) can determine positional precision and accuracy. Repeatability of the spot centers is also correlated with uniformity in diameter and indices of roundness. The data from the array in Figure 6 had an average standard deviation (SD) in position of 19 µm in the x direction of the array, and 12 µm in the y direction. The average absolute location of each spot was within 12 µm of the programmed dispense location. The image analysis program also showed that the droplets had uniform areas and circularity. Morphological features are often more important in high-density arrays, rather than the specific volume, per se.

The final requirement for biosensor production is to have a platform that can meet the production and

dispensing demands for volume and positional accuracy and precision. For example, depending on the mode of production and throughput requirements, the BioJet Plus technology can be incorporated on a variety of available platforms. Such systems as the BioDot AD3400 and continuous in-line web systems such as the BioDot RR4500 can be used for research and development and scaled through to production (see Figures 7 and 8). Components such as shuttle mechanisms, vision inspection systems, and bar code readers can also be integrated to provide a fully functional production system.

Table III shows the typical production capabilities for a range of BioDot platforms that incorporate the BioJet Plus dispensing technology. For example, using the AD3400 in a batch production mode, an IVD manufacturer can expect approximately 70 million parts per year. Using the same dispensing technology but in an in-line continuous production mode, the number of parts increases to approximately 240 million parts per year. In making the transition from research and premanufacturing to full-scale production, the only change is in the platform capacity and not the core dispensing technology. This reduces scale-up validation issues as the same technology is used in both research and production.

Product	AD3400 8 BioJet Plus dispensers single shuttle	AD6000 8 BioJet Plus dispensers dual shuttle	AD6000 8 BioJet Plus dispensers indexing web	RR4500 8 BioJet Plus dispensers continuous web
Production Format	Batch production	Batch production	Batch production	In-line production
Estimated Throughput for 1 shift (8 hours)	72 MM parts per year	100 MM parts per year	126 MM parts per year	240 MM parts per year

Table III. Estimated throughput for various instrument configurations.

Conclusion

This article addressed some of the major challenges for biosensor development and production. Key among those challenges is the reliability and scalability of the manufacturing process, which in turn depends on the design and manufacturability of the product. Working with equipment manufacturers starting from an early stage in the product design ensures that the process requirements and manufacturing equipment match.

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