
THE UP SIDE OF FAILURE

For Dan Pregibon, a failed experiment led to a major discovery: a low-cost process for printing microparticles used by researchers in **MULTIPLEXED DETECTION OF BIOMARKERS**. The tools that have evolved from Pregibon's invention promise to increase the efficiency and accuracy of detection in life sciences research and clinical diagnostics.

Someone else got there first.

A week before his Ph.D. thesis proposal was due, Dan Pregibon learned that a research paper had just been published on a topic similar to his own. Pregibon had planned to develop technology for efficiently sorting cells in the blood, based on phenotype. Now he would have to find a new topic. But first, he had to wrap up the work he'd been doing, which proved to be serendipitous.

SIMPLE METHOD, COMPLEX PARTICLES

Pregibon had been using photosensitive microfluids to create decorated gel patterns, immobilized on a glass surface, that would be used to capture cells of specific phenotypes. When he tried performing the same process on an elastomer surface, the experiment failed—the patterns floated away.

"I was fascinated," he says, "but I had no idea what it meant." In fact, he had stumbled on a streamlined way to print microparticles, free-floating microscopic structures, of virtually any shape or chemical composition. Under the guidance of his advisor, Professor Patrick Doyle of MIT's Chemical Engineering department, Pregibon worked with another lab member to develop the fundamental technology.

The team later applied the microparticles to the detection of biomarkers, such as DNA, RNA, and proteins, that can be used for clinical diagnostics. Comparable detection tools, such as microarrays, are costly to produce and require expensive, specialized scanners. So this invention could reshape the landscape. It also gave Pregibon a fruitful new thesis topic.

TWO STREAMS, SIDE BY SIDE

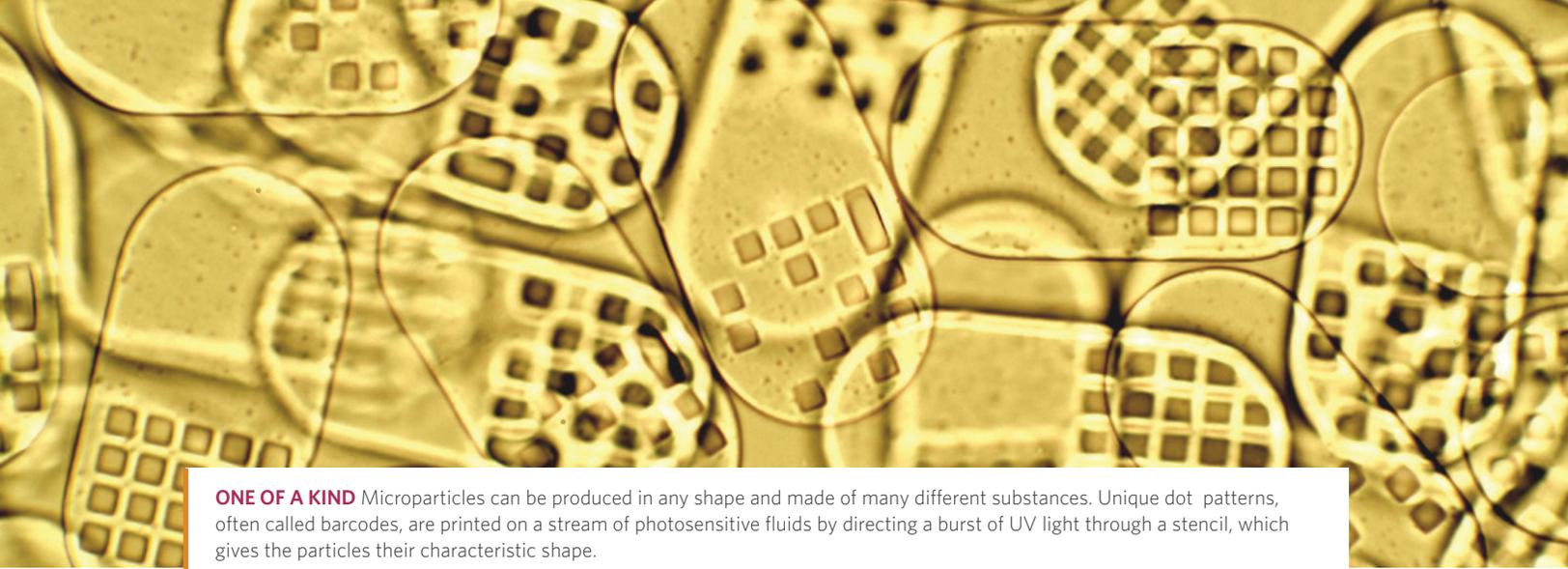
Pregibon developed a high-throughput method for printing complex microparticles on streams of photosensitive liquid. The process exploits the tendency of microfluid streams to remain distinct from each other while in the same channel. Exposed to a pulse of ultraviolet light projected through a stencil, the two streams solidify into a single particle, shaped by the stencil.

One stream may contain probe molecules for targeted proteins, DNA sequences or RNAs. The other stream is imprinted with a unique barcode so different particles can be readily distinguished from each other under a microscope.

This approach allows a scanner to accurately detect and quantify multiple targets in the same sample. It also makes it possible to combine multiple particles in custom-designed assays, based on the end user's specific detection needs.

KEY INNOVATORS

- **DAVIDE M. MARINI, Ph.D.**, Firefly BioWorks co-founder and Chief Executive Officer
- **DANIEL C. PREGIBON, Ph.D.**, Firefly BioWorks co-founder and Chief Technology Officer
- **PATRICK S. DOYLE**, Professor of Chemical Engineering at MIT, Firefly BioWorks co-founder



ONE OF A KIND Microparticles can be produced in any shape and made of many different substances. Unique dot patterns, often called barcodes, are printed on a stream of photosensitive fluids by directing a burst of UV light through a stencil, which gives the particles their characteristic shape.

A MILLION BARCODES AND A 100X IMPROVEMENT

With funding from the Deshpande Center, the team was able to validate the basic science of Pregibon's invention. They showed it was possible to create up to a million distinct encoded particles and thus detect a million different targets, all in the same sample and with a 100-fold improvement in sensitivity over comparable detection methods.

“Of everything great that came out of the Deshpande Center, meeting Davide was certainly the most valuable.”

DAN PREGIBON

The Deshpande Center also provided Pregibon with mentorship and business advice. More importantly, it brought Davide Marini into his orbit at a serendipitous moment. In 2009, Pregibon gave a talk at an IdeaStream symposium sponsored by the center. Marini, an expressive man with a warm manner, approached him afterwards and declared his work “beautiful.” The two hit it off, and Pregibon was quickly impressed by Marini's mix of business acumen and research expertise.

Marini had left a lucrative career in finance to earn a Ph.D. at MIT and then work in medical research. With support from the Deshpande Center, he had spent four years working on a technology for monitoring ion

channels, structures he calls “the biological equivalent of transistors”, only to conclude that his idea wasn't amenable to commercialization. Deeply disappointed, he had resolved to move ahead by helping someone else with their work. Almost immediately, he met Pregibon who soon invited him to join in forming a company.

In one of his most strategic contributions, Marini successfully argued that—rather than develop a purpose-built scanner—they should redesign their encoding process so their microparticles would be universally readable by standard scanners, starting with the 80,000 flow cytometers being used worldwide. Doing so would shorten the path to commercialization and create a ready market among labs that already owned flow cytometers. This strategy would also allow those same labs to try out new detection products at no added cost for equipment.

In 2009, the team co-founded Firefly BioWorks, with Marini as CEO and Pregibon as CTO. The company's first commercial offering is the FirePlex™ line of hydrogel particles for detecting microRNAs, which regulate protein expression in many human genes. The FirePlex technology can rapidly profile up to 70 different microRNAs over large numbers of biological samples. Its first uses will be in life sciences research. Applications for clinical diagnostics are expected further down the road. “Our biggest hope,” says Pregibon, “is that 10 years from now a standard blood workup will include a screen for early signs of cancer.”