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WHITE PAPER GENOMICS

Semiconductor technologies and system concepts to revolutionize genomics

Contents

Who should read this white paper?

This document is intended for anyone involved in **genomics** and new emerging domains such as **proteomics and single-cell sequencing**. It is intended for companies from the full ecosystem: **start-ups** developing new sensor concepts, manufacturers of (deep) **sequencers** and point-of-care solutions, **data** analysis and visualization experts etc.

Since 1984, imec's expertise has been in semiconductor chip technologies for a multitude of application domains, amongst which is sequencing.

Our partners in the sequencing world give us insights into the trends shaping the future of healthcare and genomics. We have translated these trends – and its associated challenges – into **a toolset of semiconductor technologies** that can act as a gamechanger for better sequencing equipment and tools.

The most important lesson to learn from the semiconductor world, is the concept of **cross-layer optimization (also referred to as system-technology co-optimization)**. Rethink a product from the level of materials to components, and finally to the full system. In doing so, you will **gain orders of magnitude in throughput and cost** and be at the forefront of the next genomics era.

So, if you are interested in **semiconductor-technology-based sequencing**, and want to discover the possibilities of nano structures, silicon fluidics, large-scale parallelism, and cross-layer optimization as a way to drastically reduce cost or footprint and to increase throughput, then this white paper is for you.

Enjoy

1. Introduction

In October 2020, the National Human Genome Research Institute (NHGRI) published its 3rd strategic vision document entitled "**Strategic vision for improving human health at The Forefront of Genomics**" [1]. With the former versions written at the end of the Human Genome Project (2003) and at the beginning of the last decade (2011), this update again heralds a new era in genomics with specific challenges and opportunities.

The NHGRI defines 4 key areas to focus on:

- enable the use of genomic medicine in the healthcare system for prevention, diagnosis, and therapy. Genomic testing will become as common as today's blood test;
- unravel the biological function(s) of every gene in the human genome, and of the non-coding regions;
- understand the full spectrum of genomic variation and find its relation to human diseases;
- develop new data-science capabilities to keep pace with the petabytes of data generation each year.

1.1 Cheaper, faster & more accurate

Both the scientific world and healthcare providers will need the appropriate tools to realize the above vision by 2030. The tools to generate and analyze genomic data should become cheaper, faster, and more accurate. From a cost perspective, the ambition remains high. After a reduction of more than million-fold – from 100,000,000 to 1,000 dollar per genome – in the past 20 years, the curve should go down further to **100 and even 10 dollar per genome** [2].

Genetic testing will make its way from the lab to the patient's bedside, with a **more compact** form factor, and with results **in real-time** or within a few hours. They will assist doctors in making the right decisions for their patients' treatment, e.g. should a lung infection be treated with an antibiotic or not? Is this COVID-19 patient infected by the British or South-African variant? Imec's vision on such a future point-of-care device is depicted in Figure 1.

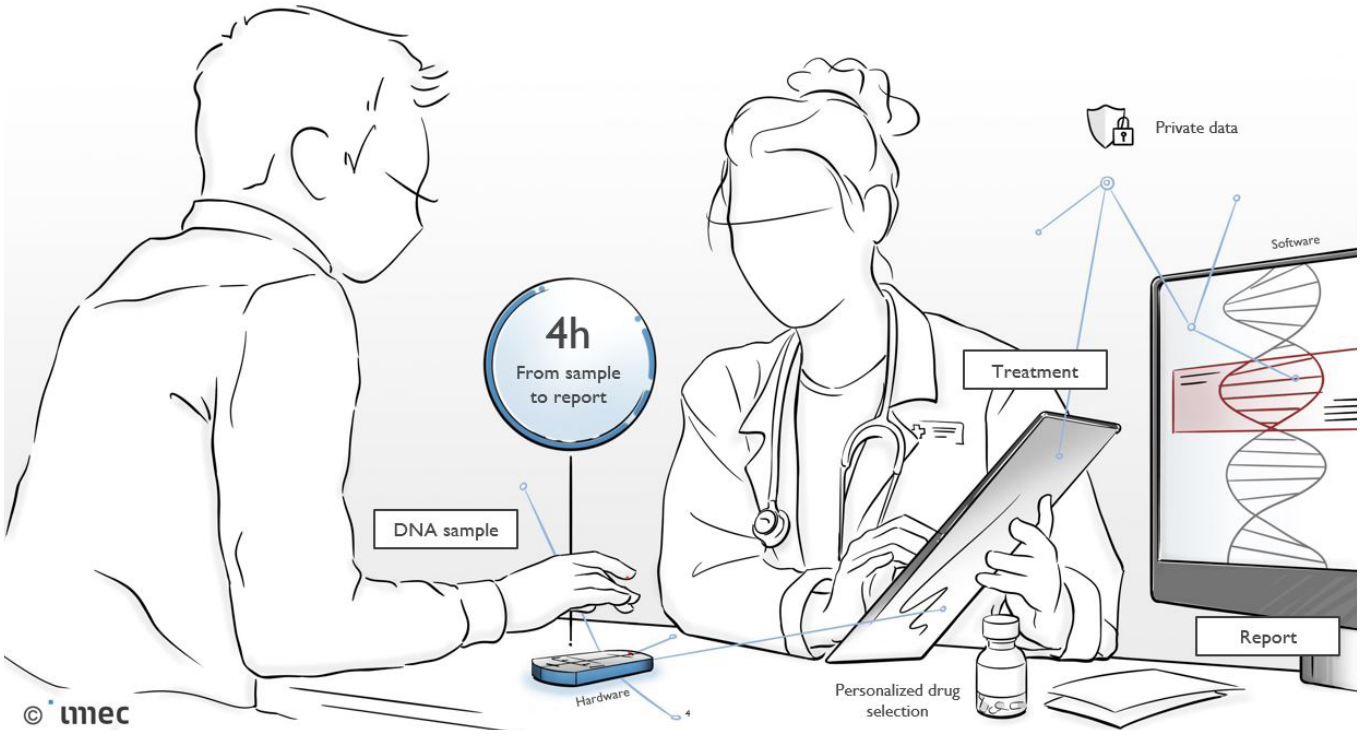

Figure 1 | Imec's vision on point-of-care sequencing: a portable connected DNA sequencer for daily clinical practice. Using cross-layer optimization, imec believes it is possible to realize a sample-to-result time of only 4 hours.

Sequencing & the pandemic

The current pandemic has learned the world that sequencing is an indispensable tool for healthcare. The sequence of the SARS-CoV-2 virus was published already 4 weeks after the first patient samples were processed and allowed the identification and development of PCR tests and the design of RNA vaccines. Moreover, sequencing is now used in daily practice to monitor the virus mutations and variants. It is an excellent example of how genetics has helped to gain efficiency in dealing with medical problems.

It is thanks to new technologies that today's sequencing solutions were able to accomplish these results. However, a virus is a relatively simple sequence of about 30 kbp coding for 27 proteins. Compare this to a human genome of 3 billion base pairs. The sample-to-result time and cost are still too high to be widely deployable for applications such as liquid cancer biopsies or single cell sequencing. The current technologies allow us to look for a few known needles in the haystack, but not yet to sequence large parts of the human genome in clinical routine. There is a need to further increase the throughput and efficiency. Semiconductor technologies will play an important role in achieving this.

To better understand diseases, **deep sequencing** tools are important. They are able to search for rare sequences, cells, or infectious agents in a sample. Innovations in this area should focus on **increased throughput**, lower cost and higher accuracy. In the end, deep sequencing will help us to better understand, diagnose, and treat cancer. It will be used for liquid biopsies and to identify mutations within tumors.

For **advanced research**, new tools will be developed that provide more information than is available today. For example, because of mosaicism (i.e. the genomic variation among cells within an individual), it is important to sequence individual cells while knowing their location in a tissue or tumor. These domains, referred to as **spatial omics and single-cell sequencing**, will help scientists to study and decipher the function of tissues and the development of cancer [3].

And finally, **data storage, data visualization, machine learning, and artificial intelligence** will be key areas of innovation to handle the skyrocketing amount of sequencing data. Also, it will enable to integrate genomics into medical practice, and to connect the growing body of genomic knowledge to clinical decision-making.

1.2 A new wind coming from the semiconductor industry

Interdisciplinary research and the recruitment of **new expertise** will be indispensable to fuel this next era in genomics, according to the NHGRI's vision document. Imec believes that semiconductor technology is a great example of such new expertise. As no other industry, the semiconductor industry has succeeded in making more complex and scalable systems, in a compact form factor and at a reduced price, with improvements year after year.

Already today, expertise from the semiconductor's industry is being used for genomics. Next-generation sequencing experienced an unprecedented performance and cost scaling. Faster even than the performance scaling in the semiconductor industry, that is projected by **Moore's law** (i.e. a law that predicts that the number of transistors on a microchip would double every 2 years due to shrinking transistor dimensions and as a result this would also double the performance of microchips in about 18 months). However, the revolutionary progress in the genomics industry is stalling lately on **a plateau of about 700 dollar per genome**.

On the next pages we will dive deeper into the possibilities of this very promising Watson & Moore marriage and how it will help build the next exciting phase of the human genomics journey in line with the NHGRI recommendations.

2. Semiconductor technologies, from sample prep to data analysis

2.1 Interaction is key when building a system

Specialization has shaped our society and economy. It's the base for progress in so many different domains. Also, the microchip industry has long relied on the immense knowledge and creativity of its design, technology and system experts, all optimizing a specific layer of the final system.

Some 20 years ago the path of '**happy scaling**' [4] or Dennard scaling came to a halt: the making of ever smaller transistors provided diminishing returns in speed, power efficiency and cost [5].

The semiconductor industry learned to take a new, wider view on its systems. When designers and technology experts talked to each other, the design could be made smarter, incorporating the pros and cons of new and older transistor technologies. The concept of **cross-layer optimization** came on scene as a new way to further improve systems in cost and complexity [6,7].

The genomics industry has also experienced its 'happy scaling' era with a more than million-fold decrease in cost per genome. New expertise and a more **holistic view** on the system and its layers will enable the continuation of this path.

On the next pages, we will discuss several **semiconductor-based concepts** that can bring value to the different steps in the sequencing workflow, from sample preparation, to sequencing and data analysis (Figure 2).

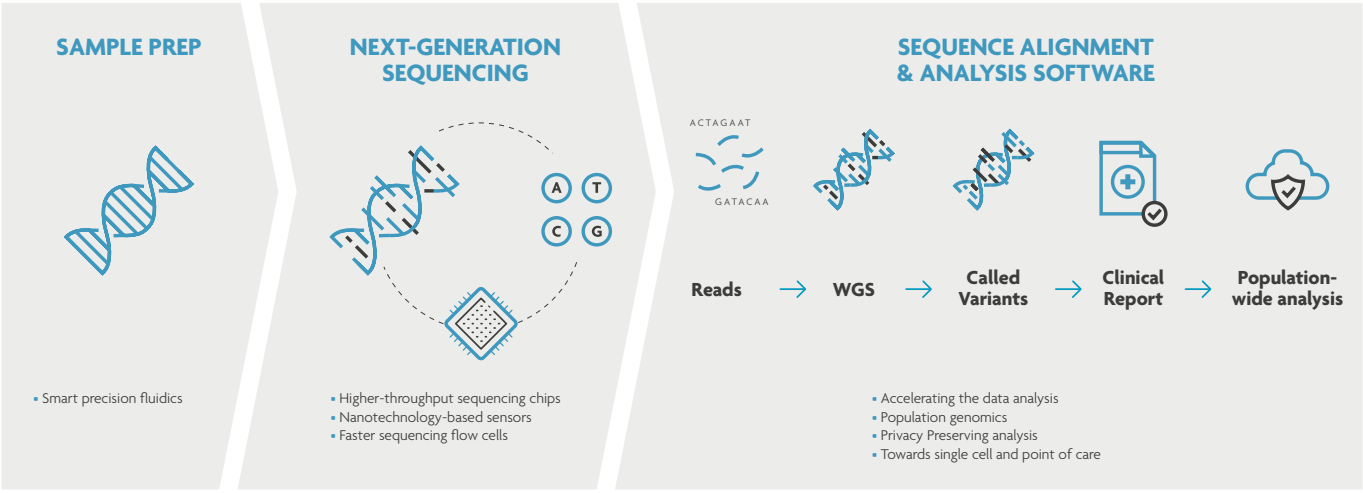

Figure 2 | Semiconductor technology can bring value to the different steps in sequencing, from sample prep to data analysis.

Imec has **experts in house** to help with many of these technologies, from material specialists to hardware and software developers. Imec stresses the importance of interaction across disciplines and tries to incorporate this in its projects. Also for genomics it could be very valuable to consider this cross-fertilization between disciplines and system layers.

Figure 3 | A typical semiconductor wafer with chips. This thin slice of semiconductor material (e.g. silicon) is used for the fabrication of electronic chips.

What is cross-layer optimization?

Traditionally, the semiconductor industry and its success were based on producing ever smaller, identical transistors. This miniaturization of transistor dimensions resulted in faster and more energy efficient systems with increased functionality and/or smaller size. Experts in materials, processes, devices, circuits, systems and software layers have been optimizing their part for many years to get the best feature size and best compute circuits and software. The best illustration for this is the evolution of the cell phone that has become a pocket-size supercomputer.

However, in early 2000 it became clear that smaller transistor dimensions would come at a price. Transistor characteristics began to vary across a silicon wafer, although all transistors were fabricated using the same process. Also, performance started to slip with high leakage currents that made it hard to design a good circuit.

Yet, the semiconductor industry succeeded in keeping up the pace of its success, thanks to a concept called 'cross-layer optimization', also know as design-technology co-optimization and system-technology co-optimization. Rather than making reliability and leakage currents solely the responsibility of manufacturing, reliability management became a cooperative effort across the system stack involving circuit design, architecture, firmware, operating systems, middleware, compilers, and application software. This cross-layer, full-system co-design approach proved to efficiently compensate for the reliability challenge. Imec believes that this approach would also be very valuable for the genomics industry.

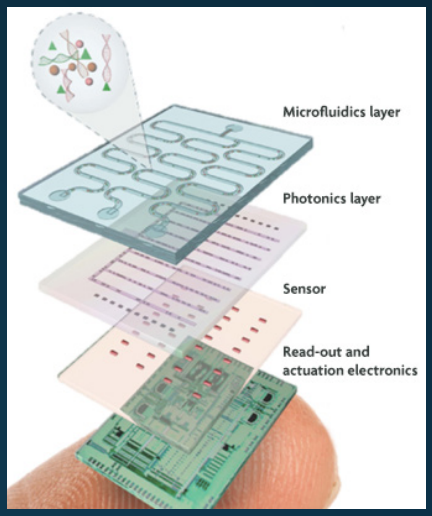

Figure 4 | In future sequencing equipment, integrated chips could contain (from bottom to top): the read-out and actuation electronics, the sensor, potentially also photonics and the microfluidics layer, where also the biochemistry reactions are taking place. All are built on top of the CMOS wafer using post-processing steps.

2.2 Chip-based sample preparation

Once a sample is collected, it needs to undergo several steps before the DNA (or RNA, protein, ...) code can be read. Today, this sample preparation requires different instruments equipped by skilled operators. This results in a lot of queuing time and a high turnaround time.

The solution to this problem is **full automation** of these preparation steps. A promising route to achieve this is by using **smart precision microfluidic** channels, structures, and concepts, both for deep sequencing and point-of-care sequencing.

From a material point of view, there are several possibilities for realizing the microfluidics. **Plastic**, which is the standard today, is an interesting choice because of its price tag. **Glass and silicon** are good candidates when high-precision structures are required and high-throughput operation is mandatory.

Future sample-preparation microfluidics will probably be **hybrid solutions**, combining the best of both worlds (e.g. a plastic cartridge channel combined with a silicon PCR chamber, requiring hybrid integration and packaging techniques).

2.2.1 Smart precision microfluidics

Lithography is used to define very precise and ultrasmall structures, also in the microfluidics part of a sequencing solution. 'Precision' means channels of a few micrometer in size, aligned to underlying sensing or actuation structures with sub-micrometer resolution. Lithography is especially powerful when it is key to make thousands of these structures to create parallelized or serialized functions to increase the throughput of the system.

Small structures also translate into **small and well-defined volumes of reagents** and **sample**, and faster chemical reactions. Moreover, semiconductor precision manufacturing capabilities enable entirely **new functions driven by microfeatures** such as micro-pillar mediated filters, sieves or extraction devices.

Realizing the sample-preparation flow with a semiconductor process has an extra advantage, i.e. a **high degree of integration** of all the components needed for a smart preparation flow. This is especially valuable for point-of-care devices with a limited form factor. One single integrated solution can e.g. comprise pumps and valves, sensors, microscopes, dielectrophoretic capture devices [8] and microprocessors for data processing.

For some functions or applications, a **hybrid solution** is a better choice to realize a cost-effective automated sample-preparation solution. This combines conventional microfluidics with precision microfluidics, sensors and electronics. This then can be integrated with sequencing chips.

2.2.2 Extraction of circulating free DNA

Circulating free DNA (cfDNA) are degraded DNA fragments in the blood plasma, originating from e.g. tumors (circulating tumor DNA) or a fetus (cell-free fetal DNA). The analysis of this type of DNA is heralded as the future of screening and early diagnosis with applications ranging **from non-invasive prenatal screening to cancer**.

The sample preparations steps for these kinds of applications include plasma separation, DNA extraction, amplification, fragmentation, library generation and clean-up, and DNA quality and quantity control.

Most of these steps can be executed in silicon microfluidics. For example, **precision micropillars** can be used for plasma separation via size filtration or DNA extraction via surface interactions, while **dielectrophoretic sorting structures** are able to isolate the relevant DNA molecules for further analysis.

DNA amplification, which is done to tackle the limited sensitivity of the detection method, can be done via **on-chip PCR** or even digital PCR amplification. While conventional methods for DNA amplification take a few hours, the above-mentioned solutions can perform the task in just a few minutes because of the small volumes and the extremely efficient thermal cycles with silicon-based chips. In Figure 5, a few of the above-mentioned semiconductor-based microfluidic structures are shown.

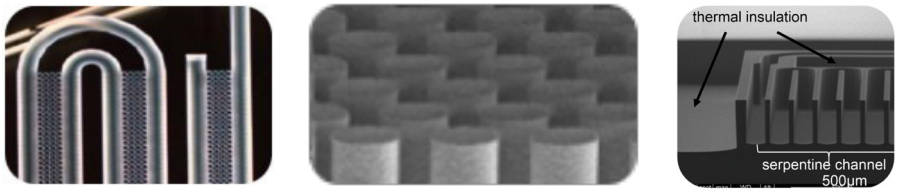

Figure 5 | Different semiconductor-based microfluidic structures for sample preparation. From left to right: crossflow filter for plasma or serum separation, micropillar filters for high-efficiency DNA extraction on plasma or serum, ultra-fast PCR microreactor (40 cycles in 3 minutes).

2.2.3 Single-cell sorting and barcoding

Single-cell genomics focuses on sequencing the DNA from an individual cell. Its applications are e.g. the study of tumor heterogeneity and the detection and monitoring of circulating tumor cells.

Sample preparation in this case focuses on the careful and efficient selection and handling of the targeted cells, and on barcoding the cells prior to sequencing. Both functions can be performed with semiconductor-based devices.

Imec fabricated a cell-sorter device that uses the creation of numerous micro vapor bubbles to create a **bubble jet flow** [9]. This mechanism is extremely gentle in moving and steering the targeted cells to a desired outlet in the flow as opposed to standard flow cytometry which operates under high shear stress with the risk of inducing changes to the cell expression profile or even impact the DNA/RNA quality.

Imec's cell sorter has many advantages: a **gentle sorting** mechanism, the potential for **extreme parallelism** with a few hundreds of channels working in parallel (and thus achieving a high throughput), and a high level of **integration** with detection (based on photonics, microscopy or impedance) and synchronization circuits (to perfectly time the cell passage and cell sorting event).

Single-cell sequencing experiments typically use short DNA **barcode 'tags'** to identify reads of single cells. In this way, all cells can be pooled together in one sequencing experiment reducing the overall cost.

Different protocols and associated microfluidic platforms exist to execute this barcoding on a sample [10]. For example, one can use **barcoded gel beads that fuse with single cells** whereafter the DNA barcode is incorporated in the cell's DNA. For this, a simple T junction is used to create oil-in-water droplets so that each cell is compartmentalized with a gel bead.

This process is **random** and it is not predictable to what extent the droplets will contain exactly one cell and one barcode. For this reason a lot of cells are lost for sequencing. Also, this method **cannot discriminate** between cells which need to be sequenced, such as cancer cells, and cells that do not, such as white blood cells.

By combining the above-mentioned **cell sorter** – to sort out relevant single cells at very high throughput – with a microfluidic T structure – to fuse barcoded gel beads with water-in-oil droplets with selected cells – and a second **droplet sorter** to sort out droplets that contain a barcode and a cell – a **very precise sample pool** can be created (Figure 6). This only contains the cells that are valuable to sequence. This helps to make the sequencing more precise and cost effective as less non-target cells and uncoded cells will end up in the sequencing pool. Especially when looking for minority cells, this cranks up the throughput and accuracy while reducing the cost.

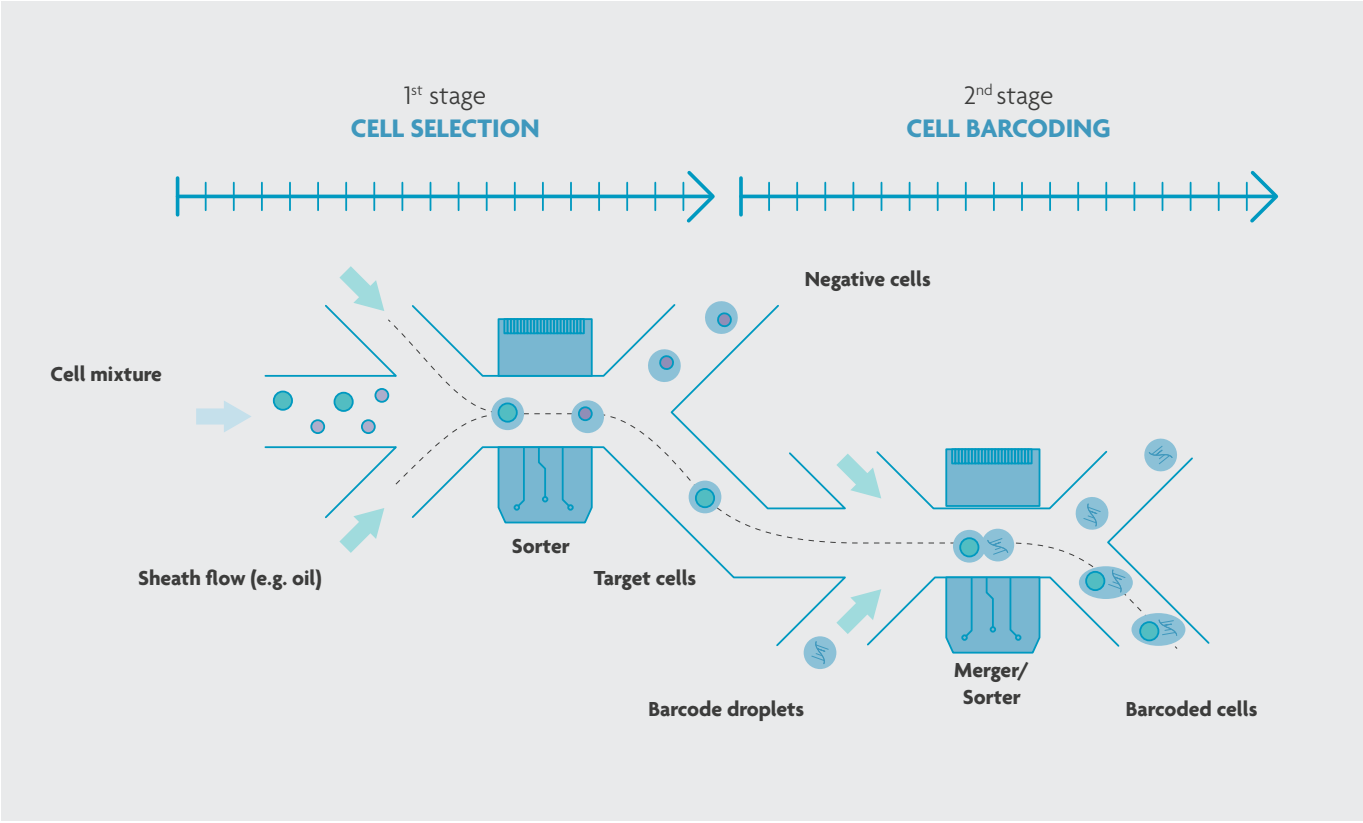

Figure 6 | Two-stage cell sorting and barcoding with a semiconductor-based cell sorter device.

2.3 Chip-based sequencing

The sequencing journey started in 1977 when Fred **Sanger** invented a DNA sequencing technique that uses DNA polymerase and chain-terminating dideoxy nucleotides to synthesize double-stranded DNA with one nucleotide length differences. Over the years, the method was improved by introducing fluorescent labelling of the nucleotides and capillary gel electrophoresis instead of slab gel electrophoresis [11]. Until the early 2000's this remained the dominant method for sequencing DNA and although genomes are now typically sequenced using other methods that are faster and less expensive, Sanger sequencing is still in wide use for the sequencing of individual pieces of DNA, such as fragments used in DNA cloning or generated through polymerase chain reaction (PCR).

In 2005, so-called next-generation-sequencing (NGS) techniques arose, based on **sequencing-by-synthesis**. Multiple detectable nucleotides adhere to a single-stranded DNA in a controlled and sequential process. The sequencing platforms became more compact and used multi-channel systems to achieve much higher throughputs than the Sanger-based equipment [12]. They did this by massively parallel read-outs of short fragments. One technology, commercialized by Illumina, focused on highly-parallelized optical detection and has been extremely successful in scaling down the cost per genome by going to very high throughput. Another example, developed by ThermoFisher Scientific, combines CMOS with charge-based sequencing-by-synthesis, thereby successfully demonstrating the sequencing of shorter fragments in less than 5 hours.

The **third generation** of sequencing is characterized by a real-time read-out, a single-molecule resolution and much longer reads. Pacific Biosciences was the first to put such a long-read system in the market based on zero-mode waveguides [13] and more recently Oxford Nanopore has launched long-read systems based on biological nanopores [14]. In the Pacific Biosciences products, the DNA is still copied, while the Oxford Nanopore solutions read the original DNA strands. Figure 7 gives an overview of this evolution in sequencing technology.

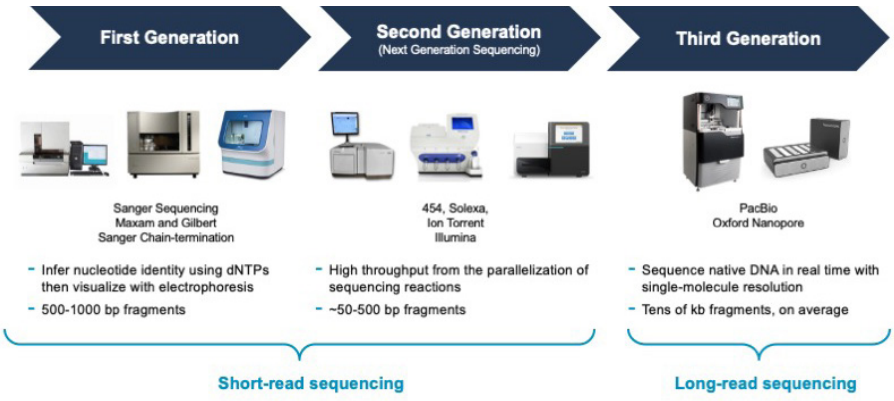

Figure 7 | The evolution of sequencing technology (source: PacBio website, https://www.pacb.com/blog/the-evolution-of-dna-sequencing-tools/)

Semiconductor process technology and CMOS technology, the most widely used fabrication process for semiconductor systems, have always been an integral part of sequencers. Just think of the large-scale patterning of flow cells, and the microprocessors and imagers that analyze, translate and visualize the raw data from the sequencing process, turning them into meaningful DNA sequences and genomes.

More recently, CMOS has taken its role one step further. One example uses ISFETs or ion-sensitive-field-effect transistors to measure the changing pH of the solution in which the synthesis reaction takes place. The ISFET 'charge sensors' and their accompanying miniature chemical reactors are **post-processed on a standard CMOS wafer** with readout electronics [15]. The tight coupling of all functionalities into one semiconductor-based system allows for dense integration and high-throughput sequencing machinery with the advantage of large-scale production in standard semiconductor foundries. Note that the data processing (microprocessor and memory circuits) is done offline and not (yet) included on the CMOS wafer.

The future 4th generation of sequencing will be marked by **more integrated sensing (electrical and/or photonic), an even more prominent role of CMOS and the shift from standard to advanced CMOS technology with nano-dimensions**. This is the key to further increase the number and density of sensing sites, and to increase system throughput and to reduce the cost, towards **10 dollar per genome**.

In the next paragraphs, we will discuss some semiconductor structures and functionalities, based on advanced CMOS, that we think are important building blocks for future 3rd and 4th generation sequencing systems.

2.3.1 Tight integration of sensors, photonics and microfluidics on a standard CMOS wafer

One of the most important and complex steps in realizing semiconductor-based sequencing solutions is the **post-processing of millions of sensors, microwells and miniature reactors** on top of a standard CMOS wafer with analogue frontends and potentially also logic and memory functions.

The most efficient and economical way to do this post processing is in a full-scale industrial cleanroom like the ones used for chip manufacturing. However, the problem with these is that the processes and operations are all standardized for high-volume applications.

Imec's **cleanroom** holds the best of both worlds: it is an **industrial** environment with state-of-the-art tools and 24/7 operation, and it is an **R&D** environment in which new materials, structures and concepts can be worked out by engineers and operators. In this way, it is possible to **bridge the gap** between research and large-volume manufacturing. First, imec engineers design, develop and produce the chip using industrial processes. Later, imec can produce the chips at high quality and in low to medium volumes. In a later stage, for very high chip volumes, **foundries can take over** and can further reduce the cost thanks to efficiency of scale. This way of working has helped many companies to develop and bring to the market their sequencing chips [16,17,18].

The **lithography capabilities** in imec's 300mm pilot line are state of the art. Thanks to a unique collaboration with the global leader in lithography solutions ASML, prototype tools and recipes that are not yet available for foundries, can be tested. In this way, imec has access to the smallest structures that are possible today.

Next to the advanced lithography capabilities, also **nano-imprint lithography (NIL)** can be used (on 200 and 300mm wafers). This is an emerging method to create replicated nanofeatures which is becoming popular in the life science community. Therefore, imec now offers both conventional lithography and NIL. In case of high-volume manufacturing, NIL is a very suitable candidate to reach a significant cost reduction of some of the genomic system components (e.g. flat optics, large substrates with high-repetition micro/nanofeatures). First, an extremely high-quality and large-area master template is produced (with e.g. DUV, EUV, or e-beam lithography), after which its nanofeatures are replicated over hundreds of substrates via the cost-effective imprint method. This processing method allows for additional freedom in the choice of substrates, such as silicon or glass. Most importantly, imec differentiates in making hybrid use of NIL on top of conventional CMOS wafers to reach < 5 micrometer alignment precision between both manufacturing methods.

As sensing and fluidic elements, imec makes **a variety of nanoscale structures** with dimensions well below 100nm and even as small as 5nm, using advanced lithography (Figure 8). **Nanoelectrodes** have been made using materials such as TiN, Pt and Ru, in a CMOS compatible way. All these materials are also compatible with liquids and can withstand the salty reagents used in DNA analysis. When fluorescent labels are used in a partner's protocol, **nano-photonic waveguides, flat optics and/or optical filters can be** integrated as well [19].

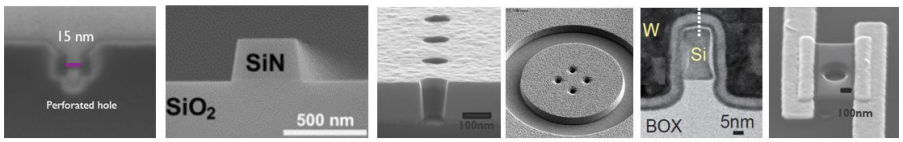

Figure 8 | During post-processing on silicon wafers, all kinds of nanostructures can be made.

Finally, **wafer-level surface functionalization** is needed for a proper functioning of the sensor (silanes, linkers, blocking sites, ...) [20,21]. The surface coatings can be patterned, controlled, and characterized at the nanoscale (Figure 9). This enables reproducible biosensing and even single-molecule deposition of DNA molecules in a controlled way. The fact that it can be done at wafer level is an important asset to take down the manufacturing cost.

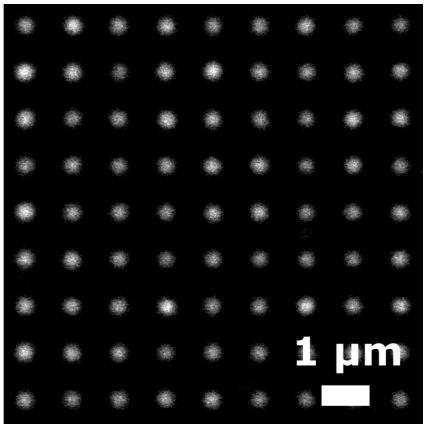

Figure 9 | Fluorescence microscopy image showing dye-labeled DNA molecules that selectively attached on nano-dot arrays (dot diameter of 250nm and pitch of 1µm). The average number of attached single DNA molecules on each nano-dot can be controlled, for example, by varying the diameter of nano-dots. This site-selective surface functionalization method is utilizing the conventional semiconductor chip patterning techniques and is thus readily scalable to wafer-level processing.

2.3.2 Bringing together nanopores and FinFETs for single-molecule sequencing

Next to this integration and manufacturing work, imec also thinks about **future 4th generation concepts** for sequencing applications. Since the first paper on nanopores in 1996 [22] it has become clear that nanopore sensors are one of the most powerful tools for next-generation sequencing because they enable single-molecule long reads and hence are key to high-throughput sequencing. Moreover, this is a more direct form of sequencing as it does not need DNA copying and in this way is even applicable to RNA sequencing.

In essence, nanopores are **small holes in thin membranes** through which a DNA/RNA molecule or a protein can move. When this translocation happens, an electronic signal is generated and this signal is indicative of the molecule's structure and can therefore be used to decode its make-up.

Nanopores can be made with **biological or semiconductor materials** [23]. The advantages of semiconductor nanopores are their superior material robustness and large-scale integrability with on-chip electronics (Figure 10). It is however very challenging to fabricate the ultrasmall nanopores and ultrathin membranes, to deal with the ultrafast DNA translocation speed and control the detection of the four nucleotides. A lot of academic demonstrations use e-beam radiation to fabricate the nanopores. This is fine for demonstrations but not applicable to large-scale and cost-effective production [24].

	Biological Nanopores	Solid-state nanopores	Nanopore Transistor
Current magnitude	Low	Low	**High**
Bandwidth	Low	Low	**High**
Nanopore noise	**Low**	High	High
Biocompability	**Good**	Bad	Bad
Multiplexable	No	No	**Yes**
Variability	**Low**	High	High
Scalability	Bad	**Good**	**Good**
Stability	Low	**High**	**High**

Figure 10 | Pros and cons of different kinds of nanopore biosensors

The new imec concept is – of course – a **semiconductor-based nanopore** and measures less than **5nm in diameter**. It is manufactured using state-of-the- art lithography equipment. This has the advantage that it can be massively parallelized to **millions** of structures on the same substrate with limited variability across the wafer. Moreover, semiconductor-based nanopores are intrinsically **very stable**.

Imec published work on **FinFETs** – the most advanced transistor industrial semiconductor manufacturing is using today – for **single-molecule sensing** and demonstrated that advanced CMOS technology nodes have promising detection limits [25]. Next, the FinFET module was extended with simulations to model charge and (Navier-Stokes) fluidics at nanoscale. This validated simulation environment has allowed to model **a novel kind of nanopore sensor** that can be produced with lithographic patterning on top of FinFET arrays [26] (Figure 11).

The uniqueness of this new concept lies in the fact that the sensing transistor and nanopore are no longer separate structures but are truly 'interwoven'. A FinFET transistor and its sensitive **gate is wrapped around the nanopore** so it can directly sense the electric field in the nanopore. The molecular signals are immediately amplified to a microampere current. The signals can be captured with more than GHz bandwidth which translates in fast reads and fast DNA translocation speeds. As a result, large arrays of nanopores can be created and can be read out at a very high speed. This concept is truly breakthrough in terms of high-throughput and low-cost sequencing.

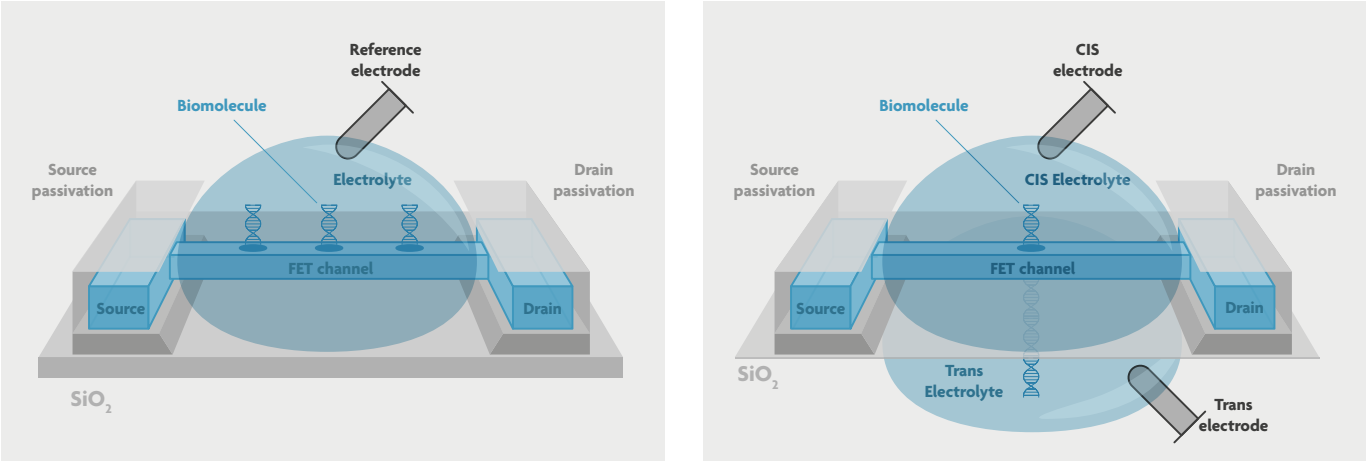

Figure 11 | Imec's concept of a FinFET (left) and nanopore (right) sensor

2.3.3 Multi-electrode chips for spatial omics

A new emerging field takes **single-cell sequencing** one step further. Spatial omics combines molecular analysis (DNA, RNA, proteins) with spatial information on **a cell's location in a tissue**. It is a revolutionary new tool for biologists to better understand tissue organization, cell regulation and disease development. Think for example of cancer: with spatial omics, the interaction between the tumor and the adaptive immune system can be studied.

A semiconductor-based tool that is very promising for spatial omics is the **multi-electrode (MEA) chip developed by imec** (Figure 12). This chip contains thousands of small micrometer-sized electrodes that each are individually addressable and can hence be used to individually measure and/or stimulate cells. The advantage of using **CMOS** technology to produce MEA chips is that much smaller electrode dimensions can be realized, achieving **subcellular resolution**. Also, since all the readout and actuation electronics (e.g. amplifiers, filters and analog-to-digital converters) are integrated in the chip, the CMOS-based MEA chips are a perfect vehicle for spatial omics. It overcomes feature limitations in the microarrays that are currently used for spatial omics. Micrometer-sized electrodes and even nanoelectrodes (~200 nm) can be easily implemented.

A tissue or tumor biopsy could be placed onto the chip so that every electrode is in contact with an individual cell. Electrodes also carry a **barcode** (DNA/RNA/protein tag), either presynthesized or synthesized on the spot using a method known as electrosynthesis. The barcodes have a location tag reflecting the electrode location. The barcode can be implanted into the cell via **electroporation**. In this case, a small voltage is applied via the electrodes to single cells to induce electroporation [27]. Next, the cells can be sequenced and via data analysis and the location tag the genetic heterogeneity in the tissue can be digitally reconstructed and overlayed with conventional pathological microscopic information.

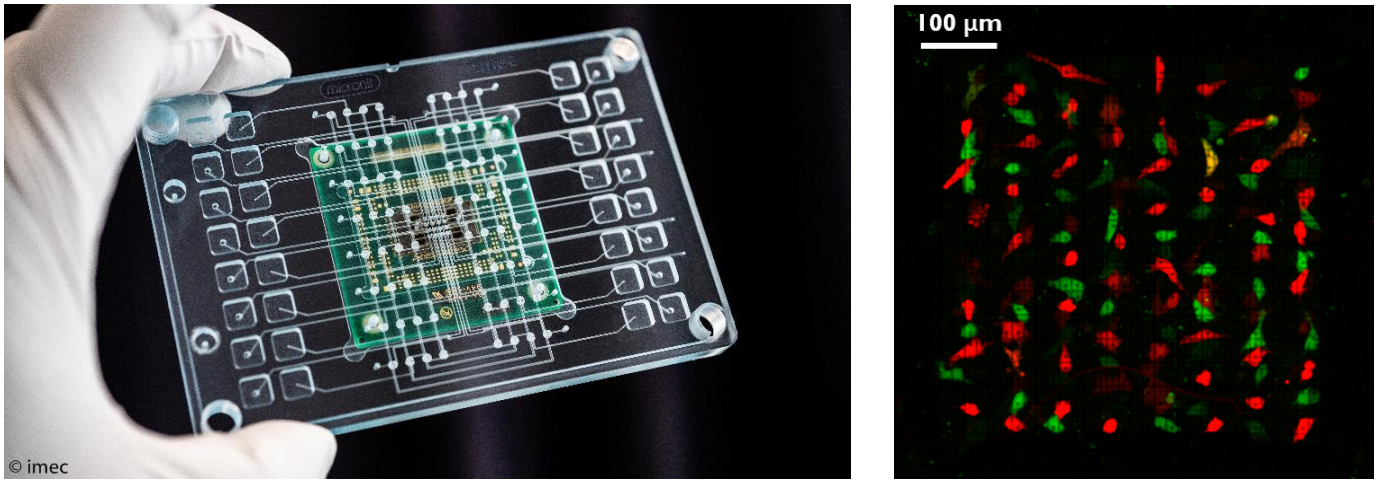

Figure 12 | Imec's multi-electrode array chip has been demonstrated to deliver two types of plasmids to a cell culture, through electroporation, with single-cell addressability. In the latter case, HeLa cells were electroporated to deliver fluorescein-labelled (green) and TRITC-labelled (red) dextrans in selected cells.

2.3.4 CMOS integration is the future

Sequencing equipment is often used in labs or dedicated sequencing facilities where tool size is not really an issue. In many cases, the readout electronics is an 'external' relatively large computer unit, packed inside the tool.

The workhorse in hospitals are flow cells that are read out microscopically and record millions of short-read sequencing reactions simultaneously [28]. This system has been used to introduce non-invasive prenatal testing in the clinic. However, for future deep-sequencing applications focus is on even higher throughput (i.e. more sensors), smaller size and lower cost.

The proven method to scale up the performance of sensor arrays is by **dense integration of the readout electronics close to the sensors**. CMOS integration is a must because otherwise the number of integrated sensors that can be read out simultaneously is limited to a few 100's limited by input/output connectors (i.e. for electrical sensors: the number of bond pads to create wire bonding). Also, the signal-to-noise and the bandwidth of the sensor is limited by the connecting leads and wire bonds.

With semiconductor-based techniques, it is possible to manufacture specially designed chips, so-called **application-specific integrated circuits or ASICs**, that contain the readout electronics, and to post-process sensors, photonics and microfluidics onto these chips. Typically, imec would do an ASIC design, a foundry would do the fabrication and imec would do post-processing on these foundry ASICS. While imec is working on novel devices for single-molecule sensing, this research goes hand-in-hand with dedicated readout electronics co-integrated into the same chip.

The design of **readout electronics, sensors and microfluidics** is tightly interwoven. To realize future sequencing concepts, experts of these different disciplines need to work together.

A good example of such **codesign** is the multi-electrode array (MEA) chip platform that imec realized for monitoring and electroporating tissue biopsies or in-vitro cell cultures [29].
These MEAs are extremely scalable with subcellular resolution, significantly higher electrode counts, possibilities to combine actuation with read out while achieving reduced crosstalk and higher functionality than passive non-CMOS MEA chips.

Readout electronics, based on 0,13µm CMOS technology, was fabricated, covering the whole chip area, and visible at the sides of the chip as shown in Figure 13. In the center of the chip, sixteen square areas with each 1024 TiN electrodes were post-processed, resulting in 16,384 sensing sites to address cells. All **16,384 electrodes** are wired to the readout electronic circuit in the lower layer of the system, making it possible to read out signals from every single electrode (and thus every single cell on top of an electrode). The rest of the chip contains all the necessary circuits to e.g. distribute the power for each and every cell to measure voltage spikes and impedance signals with attention for the low-voltage signals that have to be recorded at high speed. The chip has been optimized to read out spikes generated by firing neurons of a few microseconds and with amplitudes of a few 100 nanovolts. Also, every electrode can be individually steered to fire a signal, either a current or a voltage, to a specific cell, to induce e.g., electroporation or DNA synthesis [30]. **Microfluidic well structures** that address the different sensor regions are patterned on top in order to allow using each of the sixteen sensor areas to execute a different experiment.

A DNA/RNA/protein sequencing platform, based on a custom MEA platform, will need millions of sensing sites, making the case for **embedded electronics** very clear. The use of integrated readout puts the analogue front end and first amplification stage as close as possible to the sensor and hence minimizes lengthy leads that add background noise to the sensor signals. It reduces interference signals and is scalable. It will require **next-generation custom ASICs** gaining one to two orders of magnitude in density while the specifications for individual DNA sensors are even more challenging then is the case for neurospike recording. More **advanced CMOS nodes** will be needed, down to 22nm CMOS technologies. Moreover, when high-speed interfacing is required, such as in the case of multi-electrode real-time spike signal detection (as is the case with nanopore technology), the analogue front-end and digital read-out will need to be custom designed. One cannot fall back on existing functional building blocks such as design libraries anymore.

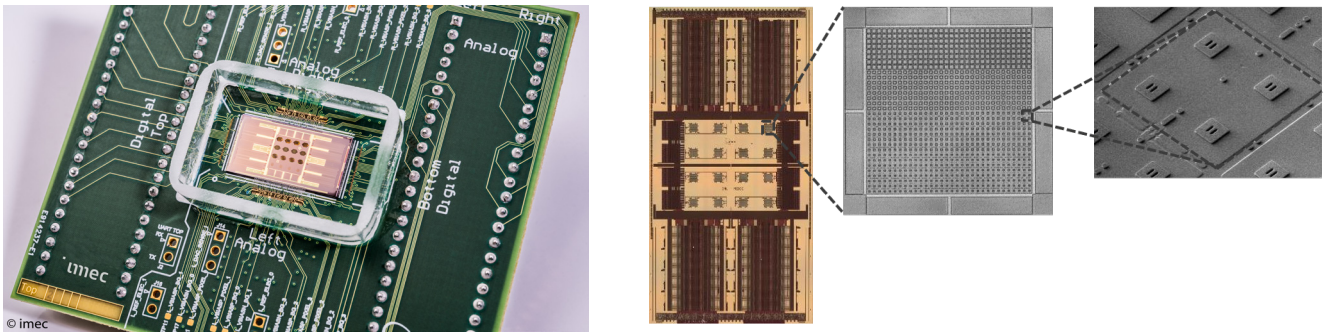

Figure 13 | Imec's micro-electrode chip with a bottom layer of readout electronics, a middle layer of sensing/actuating electrodes and a top layer with microfluidic reservoirs.

2.3.5 The final step: microfluidic assembly and hybrid integration

When the readout electronics, sensor array, photonic components and microfluidic structures are layered on top of each other, it's time for the packaging and system-integration step. To finalize the flow cell, a **cartridge with in- and outlets** is fabricated to allow interaction with the outside world. This includes electrical and photonic signals and fluid ins and outs.

The biggest challenge in this case is the **complexity and lack of standardization**. Apart from the three different input modalities, there are also the biological and liquid components inside that need to be considered. For example, the fact that the liquids may 'attack' some of the chosen (sensor and packaging) materials. Also, the biological coatings that are used for the sensor may not be damaged by outgases of used materials or by temperatures of certain packaging process steps.

There is not yet a standard sensor/microfluidic/cartridge integration solution available like for semiconductor chips. A **new platform solution** must be worked out that is sufficiently standard and can be ported to packaging industries for mass manufacturing while also being flexible to be tuned for different applications.

Imec, with both R&D, semiconductor manufacturing and packaging capabilities in house, is ideally placed to make such platform choices and to build solutions. The **large toolset of analysis equipment** is also an important asset to gain insight into pros and cons of certain material and process parameter choices, thereby removing the risk of incompatibilities at all levels. The expertise in semiconductor **3D packaging solutions** also comes in handy since it allows to develop novel technology integration solutions, based on existing successful formulas but adapted for the specific domain of sequencing or healthcare solution in general.

Three main packaging solutions exist, each with specific pros and cons (Figure 14). First, **die-to-die packaging** can be used to connect an individual chip with an individual microfluidic cartridge. Things like adhesion, sealing, and dimensional matching are to be considered. It's typically this solution that is used for first prototyping. The downside of this option is that it puts severe limits on the number of electrical and photonic interfaces to the chip and that alignment accuracies are in the order of 100µm.

	Die-to-die bonding	Wafer-level bonding	Wafer (fan-out) reconstruction
Resolution (small-sized dimensions and alignment accuracy)	Low	High	Medium
Flexibility (Si-to-cartridge dimensional match)	High	Low	Medium
Manufacturing volume	Low-Medium	Medium-High	High
Maturity	Medium	High	Low

Figure 14 | Some typical pros and cons of the different packaging approaches

Second, a **wafer-level packaging approach** can be used where chips are packaged while still on the wafer. This is interesting when there are multiple connections and when feature size and alignment are critical. For example: when a channel needs to be aligned with a sensor with < 5µm accuracy or when channels need a well-controlled depth which is easier to control on wafer level. The mass-manufacturability of this technique is very high. The wafer-bonding techniques associated with wafer-level packaging are already very popular in the MEMS world and only small variations in materials and bonding techniques are needed to make sure that the fluidics is sealed and the biosensors can be implemented. One can also fall back on the characterization techniques developed for the MEMS world such as vacuum testing of cavities to find leaks.

A challenge with the wafer-level packaging technique is however that the microfluidic package and chip should have the same size since they are bonded wafer to wafer and then diced. This is why **this solution is often combined** with the first one: precision fluidics, sealing and protection for the bioreceptors on the sensors is done by a glass that is bonded to the CMOS wafer via wafer-level packaging, in a very precise way, whereafter this glass-silicon die is bonded with polymer cartridges using the die-to-die packaging solution. This solution combines the best of both worlds: precision where needed and lower-cost injection molded or printed substrates where precision does not matter. The microfluidic interconnections to the glass-silicon die are large and can easily be assembled with the injection molded cartridges as less precision is required and process conditions are less critical. Note that this hybrid solution still has limitations in terms of number of connections because the glass-silicon chip needs to be as small as possible, and the connections dominate the footprint.

A third solution deals with this challenge: the **fan-out wafer-level packaging technique** [31]. It first slices the chip wafer into individual dies and places them on a new 'reconstructed' wafer with more spacing in between the dies (Figure 15). The connection paths on the reconstructed wafer are defined in a way that more connections are possible. Afterwards, additional microfluidics (e.g. cartridge) can be placed on top in a wafer-level style. The technique can also be used to connect multiple silicon dies, e.g. sample prep precision fluidics with sequencing sensor dies. The wafer-level fan-out solution also has cost benefits in mass manufacturing as it replaces expensive CMOS area with cheaper reconstructed wafer area. Despite all the advantages and the fact that it has endless possibilities, the technique is not yet standard and hence still costly. But like all advanced packaging techniques, it is expected that the cost will go down tremendously ones the first commercial successes take place.

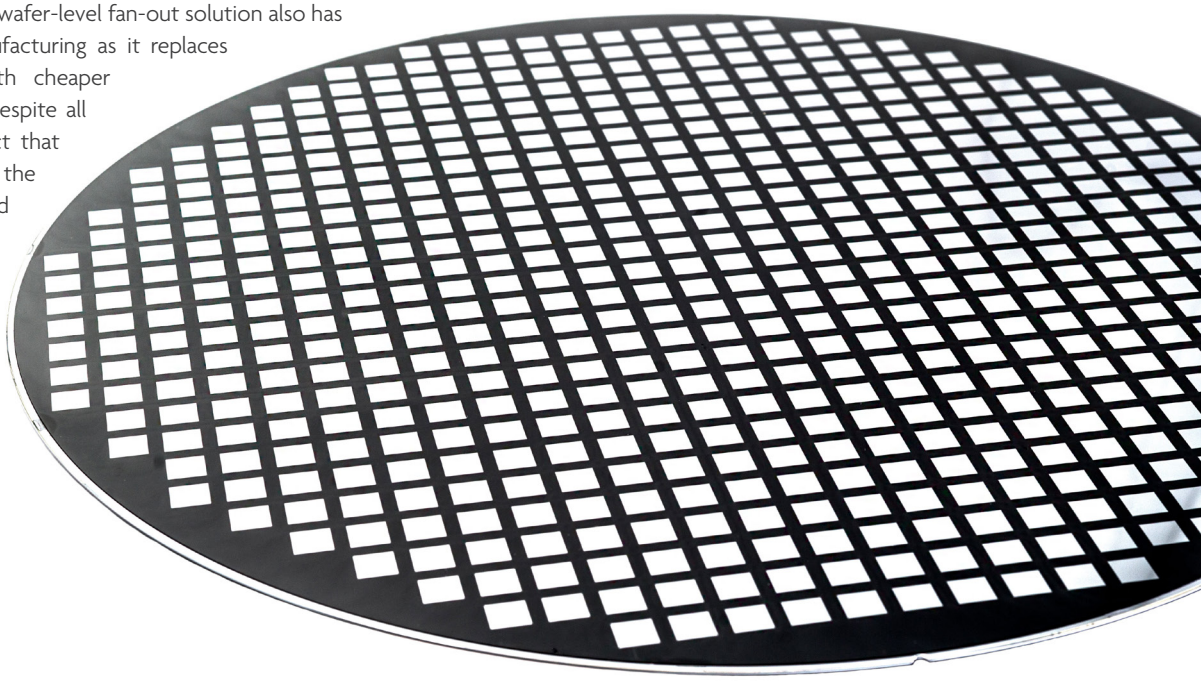

Figure 15 | A reconstructed wafer as part of the wafer-level fan-out packaging technique.

The preferred packaging solution depends on the specific application, i.e. the number of connections and (optical, electrical, fluidic) modalities, and the number of required dies. In all cases, it is key to **take the package design into account from the very beginning of the system**. The sensor design needs to use the design rules that are associated with the desired packaging solution. The importance of cross-layer optimization is extremely prominent at this final level. Figure 16 shows two projects in which imec worked out a tailored packaging solution.

Figure 16 | Projects in which the imec microfluidic 'packaging' integration expertise was used. Left: die-to-die assembled demonstrator for detection of heart rate failure by saliva (European project KardiaTool, funded by the European Union's Horizon 2020 research and innovation program under grant agreement No 768686). Right: wafer-level packaging of sensor that measures water quality.

2.4 Chip-based data analysis: sequence alignment and analysis software

Software is a key component of deep-sequencing and point-of-care tools. Smart software solutions allow to **speed up the sample-to-answer time**, and to save on the system's power. The importance of software – in the form of machine-learning algorithms and artificial intelligence – will only become more prominent in the future when the **amounts of data will explode** due to the emergence of **proteomics, metabolomics, single-cell sequencing**, etc. and when millions of sequencing tests will be performed in the daily practice of hospitals worldwide as the cornerstone of their personalized medicine practice. Patients will even have multiple tests in their life for follow-up of specific conditions.

2.4.1 Speeding up the DNA sequence analysis

After identification of the individual bases through sequencing hardware, hundreds of gigabytes of data need to be processed to **reconstruct the DNA sequence and flag variants** that might indicate genetic disorders. Today, this procedure that typically involves a series of DNA sequence analysis software tools, takes a lot of time.

Imec has set up the **ExaScience Life Lab**, that focuses on software solutions for data-intensive and high-performance computing problems in life sciences. The lab developed a **new open-source tool** for speeding up the reconstruction and variant calling process (Figure 17). In fact, the tool is 16 times faster than the genome analysis toolkit (GATK) which is the widely accepted standard reference. The significant reduction in runtime is achieved by parallelizing and merging the execution of the different pipeline steps. The tool is **named elPrep**, and recently a new version of the software was released that also includes support for variant calling [32].

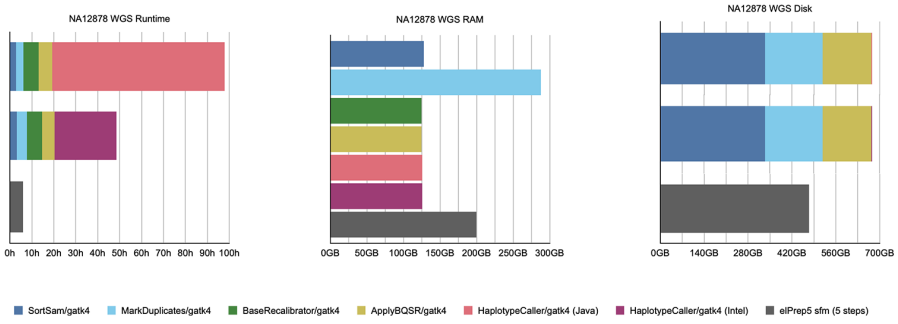

Figure 17 | Whole genome sequencing benchmark: runtime, peak RAM, and disk use in the standard reference GATK 4 (colored) vs. elPrep 5 (grey). The runtime/resource use for GATK 4 are shown per step in the pipeline, whereas all steps are combined into a single data point for elPrep 5.

The elPrep DNA sequence analysis software is validated by Janssen Pharmaceutica and Seven Bridges Genomics, and is used for production at various Belgian hospitals and companies such as BlueBee. It's written in Go, an open-source program language, and can run on any standard server on-premise or in the cloud. The elPrep software was for example used in the software stack developed in the GAP project, in which a hybrid cloud solution was realized for clinical genomics software that could operate transparently in the cloud or on-premise [33].

2.4.2 Population genomics with respect for privacy and ownership

Personalized medicine is based on population genomics, i.e. the comparison of the genomics data of millions of people to better understand health and diseases. In daily clinical practice this would mean that the DNA and patient history of a specific person is compared to the rest of the world to get answers e.g. about the type of cancer of the patient and about the best treatment.

An important hurdle in unleashing the enormous power of population genomics, is the **privacy of health data** and the fact that health info is split and **owned by all kinds of entities**: hospitals, health insurers, pharma companies, wearable companies such as Fitbit, Google, Apple, ... A free exchange of health data is therefore not possible. Also, privacy laws protect the rights of patients and do not allow entities to exchange patient data.

The solution is **amalgamated machine learning**. It enables population genomics across genome centers and hospitals, while preserving privacy of data and models (Figure 18). These privacy-preserving machine-learning algorithms can access, manage, and exchange data insights across platforms and across owners without releasing sensitive information about the internal data, nor about the models of those who own it.

Imec has experience with developing such amalgamated privacy-preserving machine learning algorithms, e.g. for pharmaceutical companies (e.g. Janssen Pharmaceutica [34]). This work was further continued in a research collaboration (VLAIO project 'Athena') involving 6 hospitals, Janssen Pharmaceutica, Illumina, RoboVision and Awell, to develop privacy-preserving clinical decision support that combines genomics and imaging data with information from hospital records to support doctors in bladder cancer and multi-myeloma [35]. Also, for sequencing companies, this concept will be indispensable to realize population genomics applications.

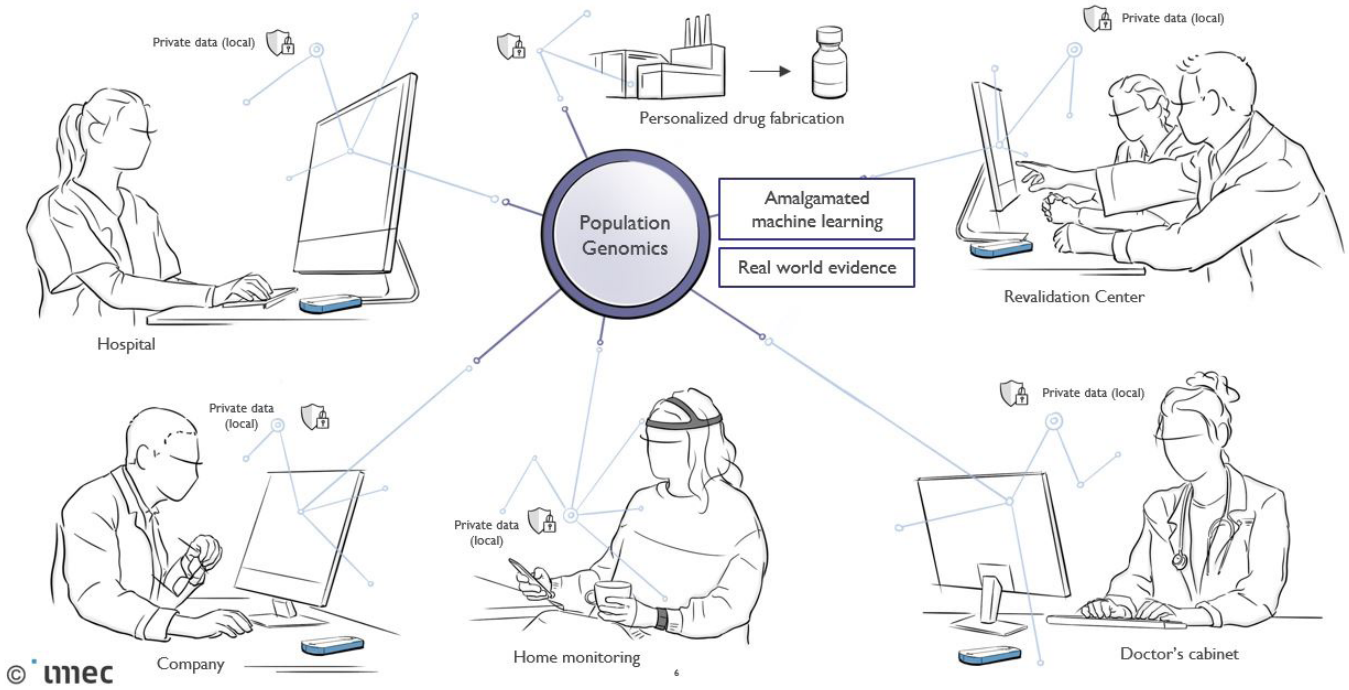

Figure 18 | Concept and vision of how amalgamated privacy-preserving machine learning algorithms can enable population genomics.

3. Future vision: a portable connected DNA sequencer

Five to ten years from now, devices will exist that – from sample to result - need **four hours or less** to transform a patient's blood or saliva sample into DNA/RNA/protein sequence information and subsequently into useful information for the patient's diagnosis or treatment.

Such **cheap, fast, and accurate point-of-care sequencing** will speed up the analysis of mutations e.g. in the domain of viromics. This is very similar to how rapid tests are being developed today, but with much more information on the virus and genetic risk factors of the patient. This makes it easier for doctors to make the right diagnosis and prognosis.

It will also open **new avenues for treatments**. Imagine performing a DNA or RNA analysis every week to track the evolution of diseases such as fast-mutating cancers. That would also mean treatments could be adapted on a weekly basis, turning what are now lethal conditions into chronic ones.

Although this vision sounds extremely futuristic, it is achievable within five to ten years from now, enabled by **cross-layer optimization**. This concept was already mentioned in the introduction of this white paper. After discussing all different semiconductor-based building blocks for sequencing applications and the power they all separately hold to improve current tools in terms of speed, accuracy and throughput, we now want to demonstrate the true power of cross-layer optimization in this visionary concept where all connecting building blocks are co-developed while taking into account all layers of the system.

The sensor that lends itself extremely well to the vision of point-of-care sequencing is the **4th generation semiconductor-based nanopore**. First, because this is a single-molecule technique and therefore the sample preparation is relatively simple and e.g. does not need any amplification steps. This as opposed to today's systems that take a prepared sample as input. Many systems are needed for library preparation and amplification before the sequencer can do its work. For future systems, based on nanopore sensors, it becomes possible to use **on-board integrated microfluidic sample prep** that consists of a simple DNA extraction and linearization using smart precision fluidics.

Moreover, semiconductor-based nanopores can be realized in **very large transistor arrays**. Nanopores are part of a transistor circuit that allows to create maximal voltage swings and hence allows to read out the sensor much faster than is the case for existing biological nanopores. **Every nanopore can have its own supporting computing and memory close by**. In this way, a fast readout becomes possible and e.g. circuits to monitor clogging of nanopores can be integrated. This will lead to more robust and reliable sequencing.

The potential of parallelization and merging of the execution of the different pipeline steps have been shown with the elPrep software which does the genome mapping 16 times faster than current tools. By co-developing this software with the nanopore sensors, the gain would be even higher.

It is important to **develop the microfluidics solution, the sensor analogue and digital readout, the nanopore sensor base calling software, the reconstruction or alignment software and the variant calling software together**, with the domain experts closely interacting with one another. In this way, a tradeoff can be made between required sensor quality and software capabilities. For example: a smarter software solution can compensate for a less accurate, and thus less complex and cheaper, sensor.

Analogue front-ends that receive the signal from the nanopore level can already do the necessary signal filtering, to realize a robust, high signal-to-noise ratio and interference-free signal taking into account the noise level that the software can correct for. Also, on-chip digital circuits can be used to realize on-chip signal compression to keep only important signatures of the nanopore spike signals and reduce the bandwidth while keeping the signals just accurate enough.

Also, with **live base calling and alignment**, and a close involvement of the software in the system, it can e.g. be possible to stop sequencing a specific part of the DNA once a good enough quality for the specific application is reached. Putting compute and memory close to the sensor is key.

Another tradeoff can be made between **local and cloud computing**. For example: variant calling might happen within the sensor very fast while for rare and more complicated cases, the full genome raw data can be sent to the cloud for a more elaborate analysis. **Amalgamated privacy-preserving algorithms** run in the background to compare the patient's data to a population of patients with similar symptoms.

Advanced **3D packaging solutions** such as (fan-out) wafer-level packaging are essential to enable the highly parallelized fluidics and the high-bandwidth electrical signals required to make this happen.

By perfectly combining microfluidics, sensor concept, read-out chip technology, packaging solution and software implementation, orders of magnitude can be won in sample-to-result time, bringing 4 hours or less time in reach. The enormous potential of this cross-layer optimization in semiconductor technology has already been proven for many other systems such as cell phones, computers, smart watches etc.

4. Conclusion

The cost of sequencing has gone down tremendously with a million-fold reduction in cost per genome. To make sequencing as common as a blood test today, or even as a finger-prick blood test for diabetes, the cost must go down even more. **Ten dollar per genome** is the aim.

Semiconductor technologies with their unprecedented capability for nano-fabrication, mass production and integration, will play a key role in this next phase of sequencing (Figure 19). For example: smart precision fluidics; cell-sorter devices; (arrays of) nanostructures; advanced transistor architectures such as FinFETs; multi-electrode chips for spatial omics; application-specific integrated circuits for sensor readout; advanced packaging techniques such as fan-out wafer-level packaging; machine learning and artificial intelligence for more efficient base calling, genome mapping and privacy-preserving population genomics.

In the 2000-2010 period, the semiconductor industry, just like the genomics industry now, started to experience problems with further scaling of costs and efficiency. It was referred to as 'the end of happy scaling'. The solution lay in the implementation of **cross-layer optimization**. No longer would electronic systems improve solely by making the transistors smaller, but instead by optimizing all different layers of the system. Experts in design, technology, integration, packaging, software etc. needed to interact and needed to consider constraints and possibilities from the next level in the system to create functional scaling.

This cross-layer optimization is also the answer to further extend the sequencing roadmap. It can enable e.g. a **portable connected DNA sequencer** that would assist a doctor in making a diagnosis or setting up a personalized treatment, a few hours after taking a sample from the patient. Tremendous possibilities await when tapping into the capabilities of the semiconductor industry.

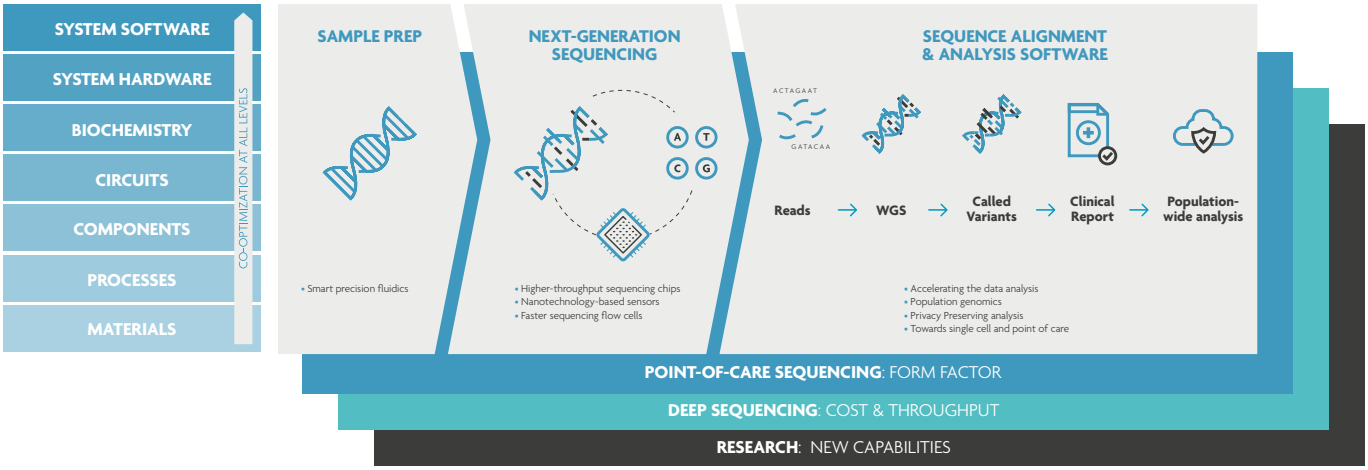

Figure 19 | The future of sequencing will have 3 main drivers, each with other key innovation areas. Cross-layer optimization, spanning all levels, will be necessary to reach these targets, and this at all steps in the sequencing flow, from sample prep to data analysis.

5. About imec

Imec is a word-leading **innovation hub in nanoelectronics and digital technologies**, founded in 1984. Its mission is to develop technology to create a connected, sustainable future for everyone. It does this together with industry and academia. More than **4,500 highly skilled researchers and staff** members from 97 nationalities work at the imec headquarters in Leuven, Belgium and in the offices and research facilities in the Netherlands, Taiwan, China, India, Tokyo, and the USA. In 2020, imec's revenue totalled **680 million euro**.

Imec is best known for its leading role in microchip technology and software and ICT expertise. The main pillars of this success are its state-of-the-art **200mm and 300mm cleanroom** infrastructure and its network of partners, covering the whole ecosystem of the semiconductor industry.

Today, imec's expertise reaches much further than the semiconductor industry, illustrated by ground-breaking innovations in application domains such as healthcare, smart cities and mobility, logistics and manufacturing, energy, and education (Figure 20).

Imec provides different business models to collaborate, such as **research programs** with multiple partners for precompetitive research, **bilateral** development contracts for custom chip design, **prototyping and low-volume** manufacturing, and licensing of mature technologies and solutions. In the domain of healthcare, imec has or is collaborating with companies and organizations such as Janssen Pharmaceutica, Pacific Biosciences, Evonetix, Roswell Biotechnologies, NASA, Agilent Scientific Instruments, Nihon Kohden, Barco Silex, the Chan Zuckerberg Initiative, and the Howard Hughes Medical Institute's (HHMI).

Since its foundation, imec has created **118 spin-off companies** and incubated 200+ start-ups. Spin-offs in the healthcare domain, based on imec technology are e.g. Bloomlife, miDiagnostics, Pulsify Medical, Onera, Epilog, and Indigo Diabetes.

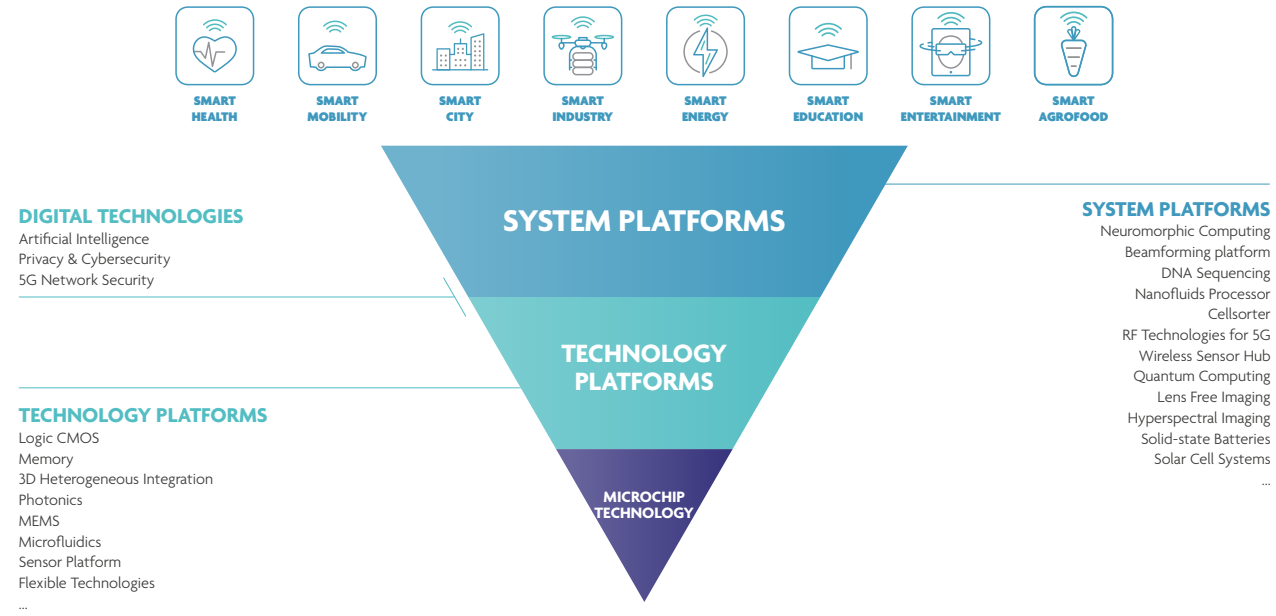

Figure 20 | Imec has expertise in a multitude of application domains, covering the full sequence from component, over technology and system.

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