Биомика, 2013, Том 5, №1-2, 62-74

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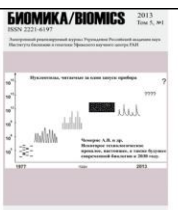

ROTHBERG SEQUENCING
(POTENTIALS FOR SEMICONDUCTOR SEQUENCING)

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Abstract
Improving the technologies of massive parallel DNA sequencing and making them more affordable in clinical studies could lead to the explosive growth of the volume of market sales of sequencers and reagents necessary for their run. The current leader on this market is *Illumina* that exploits a fluorescence-based technology for reading DNA sequences, although potential for its improvement has been substantially depleted. Semiconductor sequencing technology conceived by Jonathan Rothberg and *Ion Torrent* lags behind the *Illumina* platform by a number of parameters but develops rapidly and has a significant unused potential. This review presents a brief description of this technology and the history of its origin. A special attention is focused on unused capabilities of semiconductor sequencing technology and outlooks for its further development.

Key words: sequencing, pyrosequencing, DNA, human genome, Rothberg, *Ion Torrent*, *Illumina*, PGM, sequencer, CMOS technology, pH-sensing chip, BSI

Introduction

The first semiconductor sequencer, Personal Genome Machine (PGM) developed by *Ion Torrent*, appeared on the market early in 2011 /1/. Its initial specifications were rather modest (10-15 Mbp per run), although by 2013 its throughput is about two orders as much and exceeds 1 Gbp /2/. Sale of the next model, Ion Proton, started in September 2012. Its performance is higher (~10 Gbp per run) but the quality of sequencing in terms of read length and accuracy dropped markedly. *Ion Torrent* engineers promise that in 2014 the throughput of Ion Proton system will be more than 100 Gbp. As a result, time for sequencing one human genome should be reduced up to several hours and the cost may drop to $1000.

Figure 1 – Ion PGM and Ion Proton system semiconductor sequencers
(http://resource.lifetechnologies.com/pages2013/WE211092/images/ion_pgm_proton.jpg)

Illumina HiSeq 2500 and MiSeq fluorescence-based sequencers are still the leaders on the market. With the throughput of ~10 Gbp per run, MiSeq and Ion Proton are hot contestants, while HiSeq 2500 able to read ~100 Gbp a day is currently without a rival. generating a human genome sequence with a rather high, ~30-fold, coverage, within 24 hours and at the cost of $13,500 /3/.

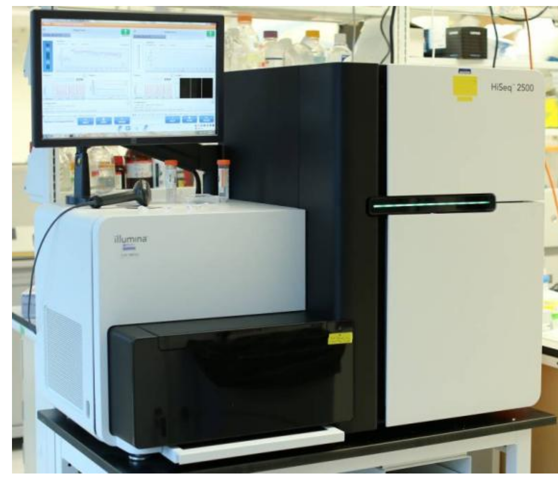


Figure 2 – HiSeq 2500 and MiSeq fluorescence-based sequencers (http://molbiol.ru/forums/index.php?showtopic=245499&st=3000)

Illumina Clinical Services Laboratory offers a 3-week express human genome sequencing using HiSeq 2500 for $11,900, and with a 3-month order the cost goes down to $9500 /4/. So, as of today (mid 2013), the price of human genome sequencing is around $10,000. The problem is that this cost has remained the same already for two years, although from 2007 to 2011 the price for one genome declined annually 2 to 5 times as much. It was because of the rapid improvement of fluorescence-based technology but its potential now is exhausted in general.

For whole genome sequencing, a semiconductor-based Ion Proton is still unable to compete with a fluorescence-based HiSeq 2500 but its potential is far from depletion. In this connection, semiconductor sequencers give to many consumers a hope that the cost of human genome sequencing declines up to $1000. This fall in cost should lead to the explosive growth of the market volume for genome sequencing and to the development of personalized medicine. When you need to perform clinical sequencing oriented to diagnostics of infectious diseases, detect disorders for a limited spectrum of genes, or determine histocompatibility in transplantology, you can use PGM, a new generation sequencer that has a lower throughput but the highest speed (~4 hours per run without sample preparation) and the most inexpensive ($49,500 without cost for server and additional equipment).

Prehistory of semiconductor sequencing

Detection of nucleotide incorporation into a growing DNA strand during its polymerase synthesis is usually used for DNA sequencing. A by-product of this reaction is pyrophosphate. In 1985, a very sensitive luminometric method for monitoring pyrophosphate synthesis was described /5/, and in 1988 Edward Hyman proposed to use luminometric detection of pyrophosphate for DNA sequencing /6, 7/. In 1997, *Pyrosequencing* (renamed *Biotage AB* in 2003*)* was founded in Sweden; its major aim was to automate and commercialize this method, but sequencers produced by this company were capable of handling a small amount of DNA templates and enjoyed no popularity.

In 2000, *454 Life Sciences* was established in the USA. Jonathan Rothberg, the founder of this company, obtained from the Swedish party an exclusive license for use of parallel pyrosequencing analysis of a great number of DNA templates (>10,000). In 2005, the company headed by Rothberg launched the world's first genome sequencer GS20 (10-20 Mbp per run). In May 2007, sequencing of the first individual human genome, that of James Watson, was completed /8/, and in June the investors participated in this development sold *454 Life Sciences* to *Roche Diagnostics* for $154.9M.

In 2008, J. Rothberg founded *Ion Torrent*. The company’s activity was focused on semiconductor sequencing technology. It is based on detection of pH changes during DNA polymerase synthesis and the

major stages of this technology are not too much different from those of pyrosequencing used by *454 Life Sciences.* This time, it took only three years to create a genome sequencer named PGM.

For the first time, pH changes during nucleotide incorporation into DNA were predicted and shown in the experiments by a Japanese group in 1992 /9/. Measurements were taken using an ion-sensitive field-effect transistor (ISFET) that recorded, as it is curiously enough, pH elevation during DNA polymerase synthesis.

In 2001, C. Toumazou and S. Purushothaman in England submitted an application for a patent proposing to add pyrophosphatase into a reaction mixture for detection of DNA synthesis and measurement of pH drop as a result of hydrolysis of the released pyrophosphate /10/. The same reaction was suggested to apply to DNA sequencing /11/, detection of mononucleotide polymorphisms /12/ and implementation of real-time PCR /13/, taking into account that pyrophosphatase in their subsequent publications and patents was not of crucial importance/14, 15/.

Some patent applications and patents concerning sequencing on the basis of measuring pH changes during DNA synthesis belong to a London-based company *DNA Electronics*, the founder of which is Christofer Toumazou. In September 2010, not longer before the production of semiconductor sequencers, *Ion Torrent* and *DNA Electronics* signed a non-exclusive license agreement allowing avoidance of possible commercial risks. Soon after that, *Life Technologies Corporation* acquired it for $725M. Jonathan Rothberg remained in the capacity of the *Ion Torrent* head and continued to improve semiconductor sequencing. In spring of this year, it was announced that *Life Technologies* would be acquired by *Thermo Fisher* for $13.6 billion, whereupon Rothberg left his post.

454 Life Sciences and *Ion Torrent* technologies

The differences between *454 Life Sciences* and *Ion Torrent* technologies at the first stage, DNA library preparation, are negligible. In both cases, the initial double strand DNA is fragmented and adaptors are ligated to the ends of its fragments providing the possibility to use one common pair of primers for amplification of different DNA fragments (Fig. 3).

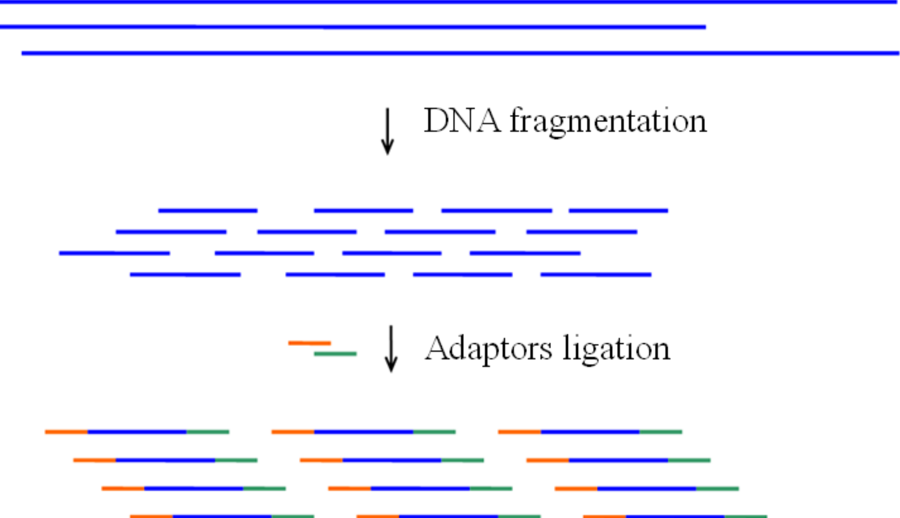


Figure 3 – A scheme of DNA library preparation

Initially, ultrasonic disintegration and even nebulization were used for DNA fragmentation. Nowadays, DNase treatment is usually employed /16/. This makes it possible to fragment DNA without enzymatic polishing of fragment ends.

After ligation of end adaptors, a fraction of DNA molecules of the optimal length is isolated from a mixture of different sized library fragments. The optimal fragment size depends on read length specific for a sequencing device used /17/. For pyrosequencing, it may be up to 1000 bp, whereas it is about 2 times less if *Ion Torrent* technology is utilized.

Concentration of DNA library fragments in the prep obtained may be very low, so for its estimation the methods of quantitative DNA amplification are better to apply /18, 19/. A precise determination of this concentration is required to proceed to the next stage of template preparation.

For DNA sequencing, the templates, i.e. identical copies of one DNA fragment immobilized on spherical granules of agarose (*454 Life Sciences*) or polyacrylamide (*Ion Torrent*) gel beads, are prepared using emulsion PCR (emPCR). To carry out emPCR, water suspension of gel granules is mixed with the DNA library and PCR reagents and, then, emulsified in oil. Concentrations of gel granules and DNA library fragments are adjusted so that a maximum amount of emulsion microdrops with one granule of gel and one DNA molecule can be obtained. One of the primers is immobilized on microspheres, that is why during DNA amplification one of the synthesized strands is also immobilized. Thanks to it, after water phase is withdrawn from the emulsion and gel granules are washed out from it, it is possible to remove the second DNA strand. As a result, gel microsphere template clones are generated, and every microsphere bears a great number of single stranded copies of one DNA library fragment.

The amount of ssDNA molecules immobilized on agarose microspheres is of the order of millions or tens of millions. The size of polyacrylamide microspheres is by an order of magnitude lower, so amount of DNA molecules immobilized on them may be tens and hundreds of thousands.

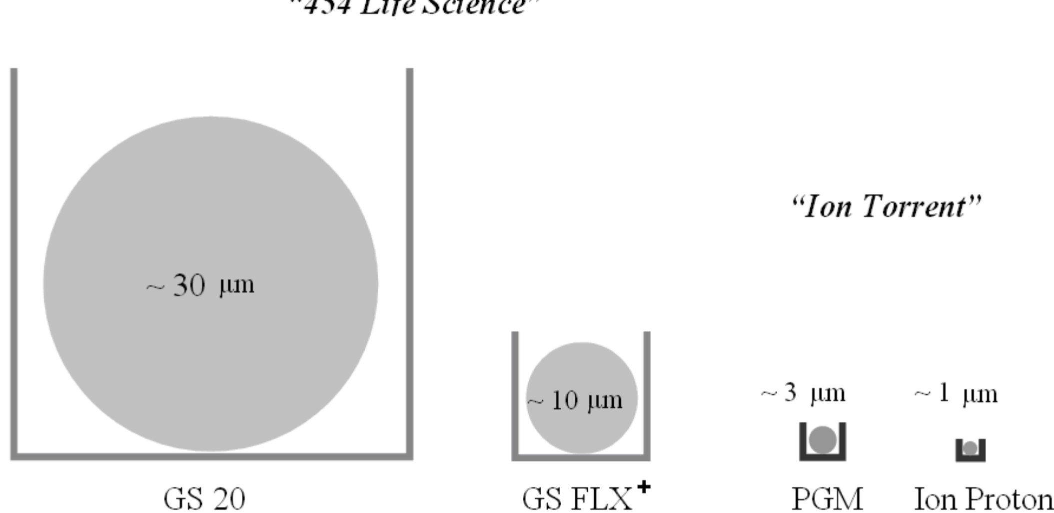


Figure 4 – Comparison of the sizes of gel microspheres

At the next step, DNA polymerase and primers, which are complementary to adaptor sites at the ends of sequenced template molecules, are added to microspheres and they form DNA polymerase complexes at 3'-ends of the DNA template. This suspension injected to a flow cell with an array of wells at the bottom. Agarose granules precipitate at the bottom of the cell and fill the wells spontaneously. For precipitation of tiny polyacrylamide granules, centrifugation is used.

The flow cell bottom size of a pyrosequencer is tens of square centimeters. The flow cells of semiconductor chips are by an order of magnitude smaller, and because of that reagent consumption is considerably lower. But the main advantage of *Ion Torrent* technology is an inexpensive cost of plain dNTPs. To detect pyrophosphate release, one should utilize additional enzymes and reagents transforming pyrophosphate to ATP (APS+sulfurylase) and ATP energy to light (luciferin+luciferase). Moreover, because of cross-reaction between luciferase and dATP, a synthetic dATPαS is required /20/ and this also raises the price of reagents.

Light from the wells at the bottom of a pyrosequencer flow cell through fiber optic light guides reaches high-sensitive CCD image sensors. In semiconductor sequencers, incorporation of nucleotides into DNA is detected by field-effect transistors of a complementary metal–oxide–semiconductor (CMOS) chip that are built-in at the wells bottom and sensitive to pH changes. In both technologies reading of the information occurs under consecutive cyclic injection of four dNTP solutions of high purity across the flow cells. Incorporation (or lack of incorporation) of nucleotides is determined by the presence (or absence) of a signal, which is light in *454 Life Sciences* or ion (H^+) in *Ion Torrent*, respectively.

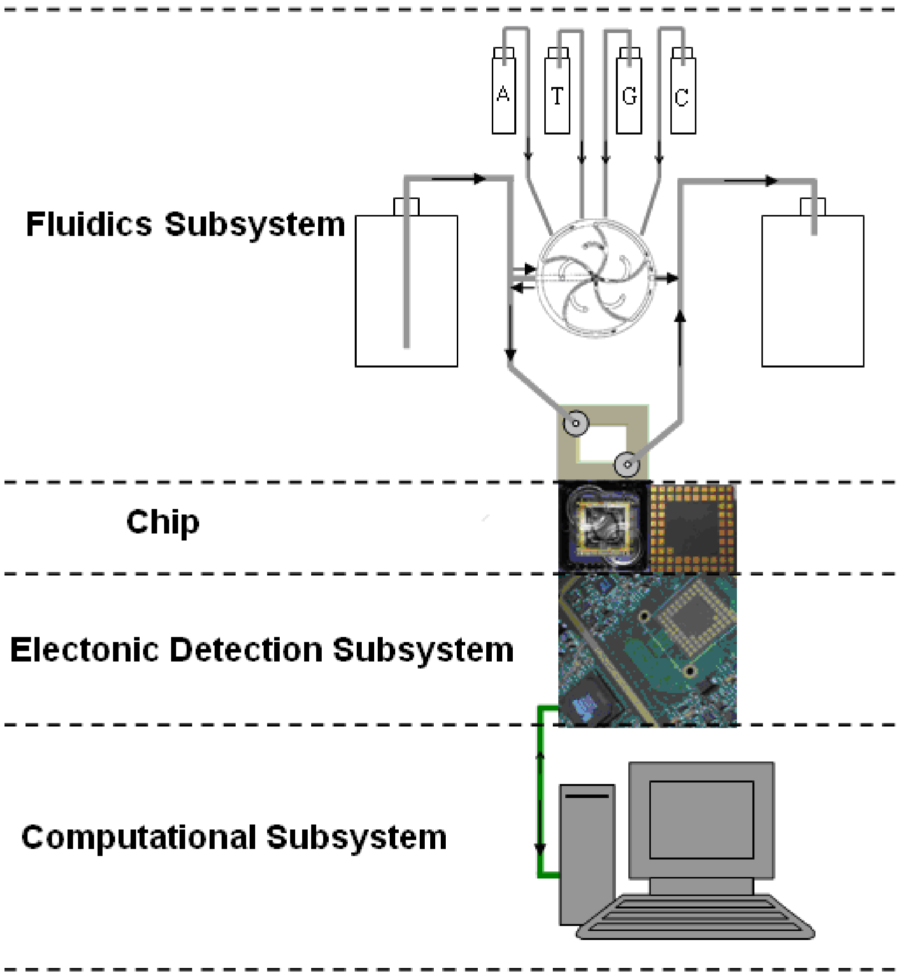


Figure 6 – Subsystems of a semiconductor sequencer

PGM and Ion Proton sequencers are designed much the same and consist of three main units including fluidics, electronic detection and computational subsystems /1, 2/.

The fluidic subsystem delivers by turns solutions of dATP, dTTP, dCTP and dGTP to the flow cell interchanging them with the washing solution. The electronic detection subsystem reads the information from the sensor array of the chip at the base of the flow cell and transmits it to the computational subsystem. Pyrosequencers are designed much alike but reusable flow cells and CCD sensors are used instead of disposable CMOS chips to read signals from the wells.

Time of one run in semiconductor sequencers depends on the read length and it is usually no longer than 4 hours. Pyrosequencers do not run much slower, but the cost of reagents is several times greater and the performance is several times lower. Hence, in competition between *454 Life Sciences* and *Ion Torrent* the latter has routed utterly the former.

In the nearest years, genome race as a competition between new sequencing technologies should overpass a cherished thousand-dollar price barrier, and it most likely happens due to improvement of the semiconductor sequencing technology. Besides, Jonathan Rothberg is unemployed again and ready to establish a new company.

pH-sensing chips

The technology of semiconductor sequencing is based on the use of disposable pH-sensing chips in which the upper (sensor) side, containing millions of ISFETs, serves as a lower side of the flow cell. The first batch of the chips developed by *Ion Torrent* for PGMs included three designs differed by the number of sensor pixels

(i.e. resolution): Ion 314 (1.5 Mp), Ion 316 (7.2 Mp), and Ion 318 (13 Mp). A part of the sensors are beyond the limits of the flow cell, thus the working resolution of the chips is a little bit lower, being approximately 1.2 Mp for Ion 314, 6.3 Mp for Ion 316, and 11.3 Mp for Ion 318 /2/.

There are 65 contact arrays at the one-inch base of the square ceramic carrier of the Ion 31x CMOS chip series. The contacts of the chip crystal are attached to the carrier contacts with thin conductors. The size of the carrier and position of the contacts match one of the CLGA (Ceramic Land Greed Array) standard variants. The wells on the sensor surface of the chips have slightly conic form. Their diameter in the middle part of the Ion 31x chips is equal to ~3 µm.

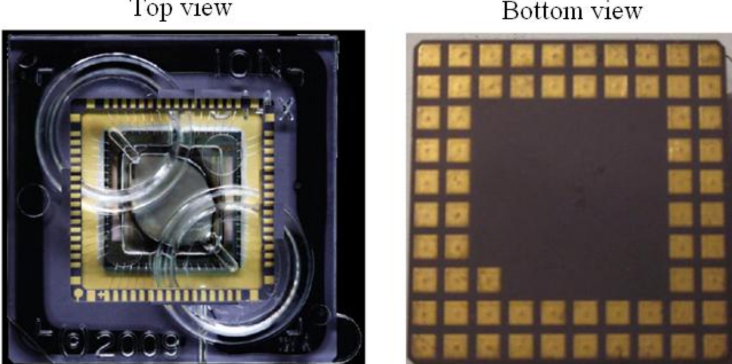


Figure 7 – Ion 314 chip

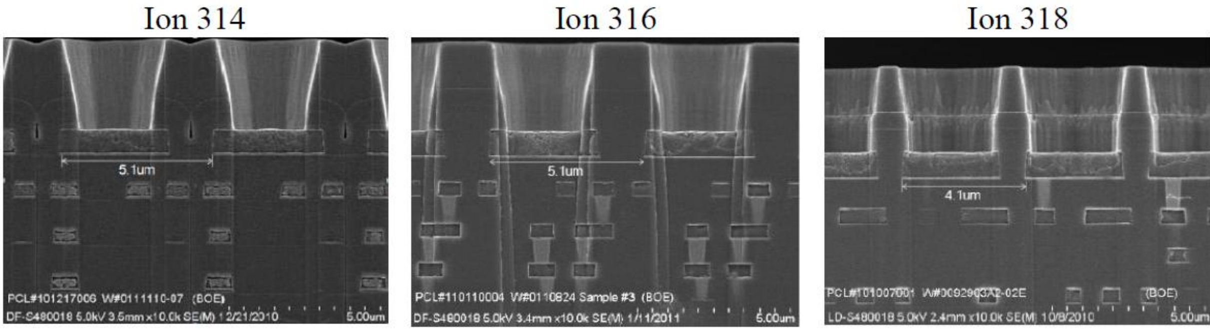


Figure 8 – Form of the wells in chips of the Ion 31x series /1/

The number of the detected nucleotide sequences, i.e. reads, as a measure of sequencer performance, is roughly two times lower of the chip resolution. This is determined by the fact that no more than 80% of the wells are usually filled with microspheres. Moreover, a part of the wells are sorted out due to nonhomogeneity in terms of the quality of the produced template microspheres or because of their pairwise penetration into the wells. As a result, at the read length of 200 bp the PGM sequencing throughput when using Ion 314 is about 100 Mbp, and with Ion 318 it may reach 1 Gbp. Recently, *Ion Torrent* began production of new chips with improved working characteristics (version v2) and increased the average read length from 200 up to 400 bp. This enabled getting a doubled PGM performance showing up to 2 Gbp with the Ion 318 chip v2.

Ion Proton sequencer is designed for the work with a different type of the chips, Ion P. Their resolution is by an order of magnitude better than that of the Ion 31x chip series. Supplies of the Ion PI chip (165 Mp) started simultaneously with the beginning of sales of the sequencers in September 2012. At the moment, it is replaced by an improved variant - Ion PI v2. In the coming months, the Ion PII chips (660 Mp) are expected, and the Ion PIII chips (1.2 Gp) could appear in 2014.

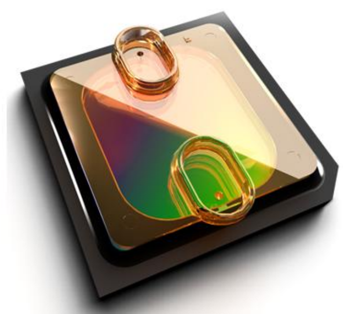


Figure 9 – Ion PI chip
(http://products.invitrogen.com/ivgn/product/4482321)

In the Ion PI chips, the wells diameter equals to 1,25 μm, and in the Ion PII it should be two times smaller. An increased number of sensors on the chip by means of their smaller size has resulted in a decrease of the quantity of DNA template molecules on the microspheres. It has also lead to an impairment of microsphere loading into the wells, additional diffusion losses of protons produced in the wells, decrease of the pH shifts and, accordingly, weakening of detected signals and worsening of sequencing quality. Thus, the average read length on the Ion PI chips did not exceed initially 100 bp and only with the Ion PI chips v2, it approached 200 bp making the Ion Proton performance up to 10 Gbp.

Dependence of the semiconductor sequencing resolution on the sizes of template microspheres and matching wells may complicate the development of the gigapixel chips, Ion PII (0.66 Gp) and Ion PIII (1.2 Gp). This dependence may lead to a lower read length and deterioration of the DNA sequence reading quality at transition to submicron sensor arrays. Nevertheless, an improved Ion Proton output, with up to 100 Gbp per run, i.e. at the scale required for sequencing one human genome, looks quite affordable, especially if we take into account that *Ion Torrent* has not used yet all possible ideas to improve chips, equipment and consumable agents for the newest technology of semiconductor sequencing.

Semiconductor pyrosequencing

Due to high rate of the proton diffusion and rapid dissociation of the proton-binding hydroxylic groups of the ISFET sensor coating, the period of the detected pH change is measured by a fraction of a second. Therefore, lowering of the frequency of sensor scanning at an increase of the chip resolution (Ion 314, ~60 fps; Ion 316, ~15 fps; Ion 318, ~10 fps), caused by the limited speed of the detecting subsystem and the chip parameters, leads to deterioration of the sequencing quality.

One of the ways in solving this problem may be transition from identification of pH changes to measuring the release of pyrophosphate (PP_i). For this purpose, on the outer surface of ISFET dielectric layer it would be required to immobilize receptor molecules, which specifically bind negatively charged molecules of PP_i. Specificity should be very high since the sensors should not react with triphosphates in dNTP, the end part of which differs slightly from pyrophosphate by the structure.

Several types of indication reagents are known that enable determination of pyrophosphate in the presence of ATP and other admixtures typical for biological samples /21/. They are designed mainly for clinical tests, diagnostics of pyrophosphate arthropathy, etc. The most specific to pyrophosphate are complexons that contain Cu /22/ or Zn /23/. Fluorescent indicators of such type are supposed to be used for recording of DNA synthesis in real-time PCR /24/.

In several patents and patent applications, Jonathan Rothberg and co-authors proposed to use for semiconductor DNA sequencing such symmetric complexons that bind specifically PP_i (US Pat. Appl. 7948015, 8349167, 8317999, 8313625, 8313639, 8306757, 8269261, 8263336, 8264014).

A possibility to detect pyrophosphates by means of micron-sized ISFETs was demonstrated experimentally in 2011 /25/. In that study, di-(2-picolyl)amine (DPA), forming a double complex with Zn cations, served as a PP_i sensitive chelator immobilized on the sensor surface. An addition of pyrophosphate caused a reversible change of the threshold ISFET current. In 2012, the same authors demonstrated that DNA polymerase reaction could be detected *in situ* by the release of pyrophosphate using ISFET with active surface 1 to 2 μm wide and 10, 15 or 20 μm long. To remove the bound pyrophosphate and regenerate the sensor surface, the transistors were washed with 0.1M acetic acid solution /26/.

A slower dissociation of pyrophosphate bound with the sensor surface enables to increase the time

period for detecting nucleotides embedding into DNA and to decrease the effect of diffusion losses typical for pH-sensing technology of sequencing. In perspective, this may lead to development of semiconductor pyrosequencers working with gigapixel arrays of submicron-size PP_i sensors. The productivity of these devices can be measured by hundreds billions of base pairs.

Specific features of CMOS sensors and sensing chips

Modern CMOS technologies are well developed and aimed at mass production of inexpensive microchips containing arrays of planar ISFETs. Extraordinary light sensitivity of these transistors allowed to develop CMOS image sensors, which make possible to get digit photos of high definition with the help of smartphones, cell phones and digital cameras. These CMOS chips contain millions or tens millions of active pixel sensors (APS), each of them consisting of a semiconductor photodiode and readout electronic circuit. Image sensors detect changing of electric conductivity of planar photodiodes when photons hit on them. In ISFETs, electric filed of the ions, sorbed on ion-selective surface of the transistor, influence electric conductivity /27/. One of the *Ion Torrent* targets was to elaborate chips with millions of APS that, instead of photodiodes, would contain ISFETs responding to changes of H^+ concentration.

Single ISFETs have long been used for pH measurements, however for mass production of rather inexpensive chips containing millions of these transistors, one cannot do without CMOS technologies. The problem is that the sensor surface of ISFET should contact the analyzed solution, whereas in CMOS technologies the transistor layer is formed on silicon plate and the access to it is blocked by the upper layers of the structured metal and dielectric films.

One of the ways in solving this problem was described by a Spanish group in 1999 /28/. They suggested forming a metal bridge in the layers of the films between the solution and the silicon base. Change in charge of the ion-sensing zone attracts or repels the electrons in the bridge and influence the charge of the control gate of ISFET placed under it.

The main technological principles for producing these multi-pixel pH-sensing CMOS chips were developed by a group of experts in Glasgow /29, 30/. Its head Professor D. R. Cumming was invited to be a member of the *Ion Torrent* Board, and one of the leading specialists, M. J. Milgrew, became head of the group of CMOS chip designers. Thanks to the British experts, a Spanish idea was realized in an American company. The design of CMOS sensor field-effect transistor with a through metal bridge turned out to be quite efficient, but in recent years an overturn occurred in the area of designing CMOS image sensors. A similar overturn is likely to be in design of pH-sensing chips. It is a matter of BSI technology /31/.

In ordinary front side illuminated (FSI) CMOS image sensors, the light sensitive layer of silicon is placed beyond a net of thin-film elements of a microchip. The abbreviation BSI stands for “back side illuminated” and means that the silicon layer is turned to the light. This inversion leads to a great improvement in a number of technical characteristics of the light sensitive pixels. Because of the absence of light losses typical for FSI sensors on multilayer electronic components of CMOS chips, BSI sensors have a heightened photosensitivity that also enables to increase the number of layers on the silicon base, improve the structure of active pixel sensors, and reduce their size.

The advantages of BSI technology is known long ago but until recently, it has been used mainly at production of expensive CCD sensors for scientific purposes. Cheap and accessible for wide use CMOS sensors of such type appeared on the electronic market only in 2010 and they now replace quickly general FSI sensors in new models of digital cameras, camcorder, notebooks and smartphones. The problem is that BSI-CMOS technology was mastered only by several largest companies and corporations, for which a rather small market of pH-sensing chips is of no commercial interest. And without their involvement, the development of BSI chips designed for semiconductor sequencing can be in fact impossible.

There are some other approaches to creating CMOS image sensors with the surface location of semiconductor layer. For example, *InVisage* is engaged in engineering sensors in which the solution of semiconductor polymer with quantum points is spread on the surface of a silicon plate with applied on it thin-film elements and upon drying it forms on it a light sensitive layer 1,5 μm thick /32/. *Panasonic* and *Fujifilm Corporation* are jointly creating chips with an organic photoelectric conversion layer applied on CMOS sensors by lamination /33/.

Most likely, the development of new technologies for production of image sensors and their use for manufacturing ion-sensitive CMOS sensors will let performance capabilities of semiconductor sequencers be improved considerably.

Potentialities of chip regeneration

The sensor coating in the first Ion 314 chips was a film of silicon nitride (Si_3N_4) but soon it was replaced with the tantalum oxide (Ta_2O_5) film that is more stable in aqueous solutions /34/. The latter facilitated a better sensitivity of field-effect transistors of the sensor to the changes in proton concentrations. The chips can be

reused due to high stability of such coating, but the technologies for their regeneration (removal of the used microspheres from the wells) have not yet been developed and would hardly be developed by *Ion Torrent*.

The main reasons of the lack of interest in chip regeneration are a comparatively high cost of DNA preparation for sequencing and a lower cost of chip production by the standard CMOS technology from silicon wafers. The cost of finished wafers (200 mm in diameter) is 100 to 500 dollars. The price of such wafer depends on the complexity of technological process, and the quantity of individual crystals obtained from this wafer depends on their size. For example, 200 to 300 crystals (10.6 x 10.9 mm) can be obtained for Ion 314 after dicing. Encapsulation of crystals and the packaging of chips may increase their cost from 1-2 to 3-4 dollars. With such a price, the reuse of chips makes no sense; however, gigapixel chips may cost hundreds of dollars and their repeated use can substantially reduce the cost of DNA sequencing.

The cost of *Ion Torrent* chips is obviously overstated: $99, $299, $499 and $699 for Ion 314, Ion 316 and Ion 318 and Ion PI, respectively. This may be a good stimulus for development of technologies for their regeneration, especially as the sensor coating of these chips (Ta_2O_5) is characterized by exceptionally high chemical stability and is able to endure even the etching with aqua regia.

Fluidic subsystem

The volumes of dNTP solutions delivered by the fluidic subsystem into the flow cell are measured in tens of microliters, while the volumes of the washing solution are measured in milliliters. The flows of the solutions are switched over by a computer-controlled selector valve, the complex structure of which provides the washing of valve channel "dead zones".

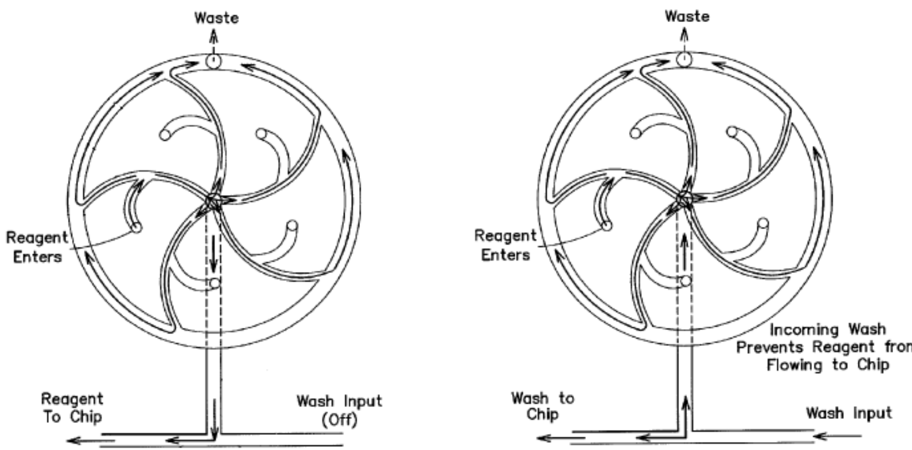


Figure 10 – Selector valve operation scheme /35/

The unit that switches over the feeding of solutions has pneumatic valves because solenoid or piezoelectric valves may be a source of interferences for the detecting subsystem. Reagents are also fed pneumatically due to the high pressure created in reservoirs with the solutions. Therefore, a bulky gas cylinder with a pressure reductor is needed for sequencer operation.

Initially, compressed argon gas was used for PGM; the application of compressed nitrogen gas in both instruments was started after the release of Ion Proton. This is caused by the fact that, under other equal conditions, the water solubility of nitrogen compared to argon is about 3 times lower, thereby resulting in a reduced emission of gas bubbles in the flow cell of the working chip heated to 55°C. However, this substitution could not overcome the "decompression sickness" that noticeably affected the quality of sequencing.

Helium has for a long time been used in the breathing gasses for deep diving to control decompression sickness. It can also be used to prevent bubble formation in flow cells of the sequencer. The reason why *Ion Torrent* does not recommend to substitute helium for nitrogen seems to be its lower availability for consumers in some countries (not including Russia).

The place of pneumatic fluidic subsystem can be taken by systems with the rotary selector valves (distributors) that have no dead zones. Such valves are usually equipped with computer-controlled stepper motors.

Valco Instruments Company Inc., USA

Rheodyne (IDEX Corporation), USA

Figure 11 – Rotary selector valves with a stepper motor driver

In addition, there are several varieties of the Lab-On-Valve (LOV) system, where the selector valve and solution feed pump are also controlled by a computer with special software /36, 37, 38/. LOV systems are highly compact and contain all elements necessary for the fluid subsystem of the sequencer. Though, some minor changes are to be made in their construction: the number of switchable channels should be increased and the measuring flow cell built in the same valve should be replaced by the output channels for connecting the flow cell of the sensing chip.

The French company *Fluigent* has recently started to produce constitutive elements for the assembly of reagent feed systems for the flow cells of sensing chips for different purposes. The ESS™ (Easy Switch Solutions) platform proposed by this company includes a 10-channel rotary selector valve (M-SWITCH™), on/off solenoid valve (2-SWITCH™), and SWITCHBOARD controller designed for connection of four rotary and eight solenoid valves. This platform is controlled by the special software MAESFLO. In addition, *Fluigent* offers its own systems for pneumatic feeding of reagents (MFCS™) and for controlling their consumption (FLOWELL).

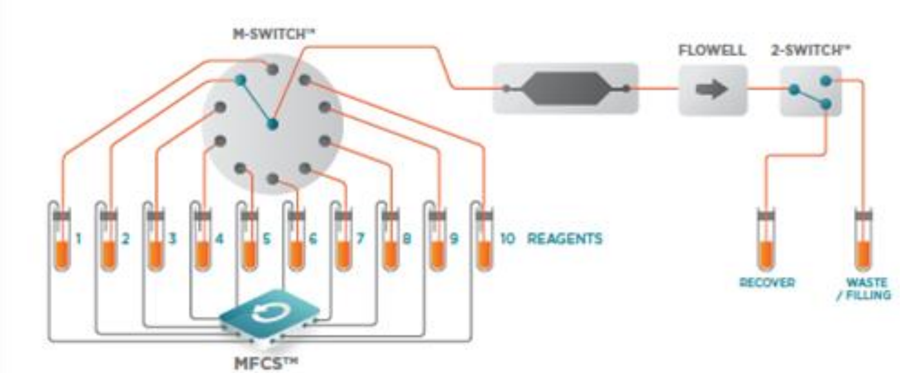


Figure 12 - ESS™ platform of *Fluigent*
(http://www.fluigent.com/wp-content/uploads/2013/07/Microfluidic-schema-switch_ESS.png)

The recent rapid development of microfluidics gives us hope that soon there will appear more compact systems for solution feed control, and the pneumatic valve system with compressed nitrogen gas will look anachronistic. Then, the press reports on J. Rothberg's participation in the development of compact autonomous sequencers for the mission to Mars and for the search of extraterrestrial life will not seem fantastic /39/.

Problems of informatics

Since the launch of semiconductor sequencers, their performance has become for two years and a half almost by three orders of magnitude greater (from 10 Mbp up to 10 Gbp) but now the rate of growth markedly dropped. Nevertheless, the probability of getting with these instruments the throughput of 100 Gbp per run is beyond doubt, even if it may take 2 or 3 years for implementation. The growth rate, most likely, will then

follow Moore's law named after one of the founders of Intel Corporation and the owner of the first genome deciphered using semiconductor sequencing technology /1/. According to this law, the density of transistors in computer chips doubles every two years. At about the same pace, we observe a rise in computer power, capacity of information storage devices, resolution of image sensors, the rate of information delivery, etc..

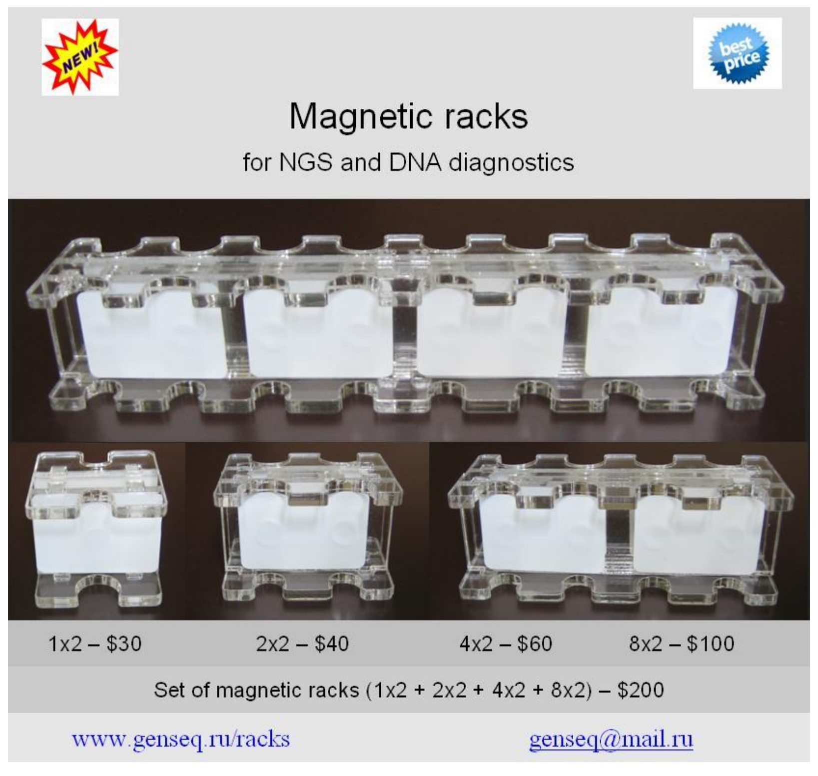
One of the consequences of this progress is that the electronics in modern scientific devices and computers that control their run become obsolete just in 2-3 years. For instance, in PGM sequencer the detection subsystem operates with FPGA (Field-Programmable Gate Array) chip ECP2M from *Lattice Semiconductor* that was rather perfect in 2008. Currently it is not the best choice.

To transmit the information from the detection subsystem to a server of the computational subsystem, PGM uses interface GigE with operating speed of 1 Gb/s. Now, instead of it, you can use interface 10 GigE (10 Gb/s) or just enjoy a widely distributed USB 3.0 (5 Gb/s). Recently, there appeared FPGA with embedded USB 3.0. In less than a year *Intel* will start to furnish home computers with Thunderbolt 2 interface capable of transferring data at the speed of 20 Gb/s.

The main part of a computer system in the first PGM sequencer series was graphic station from Dell (Precision T3500) with maximum capacity of the operating memory (24 Gb), four HDD 2 Tb each, and Intel Xeon W3670 6-core processor. Its current production is ceased and servers T7500 supplied with recently launched PGM are equipped with two 6-core processors and 48 Gb of the operating memory. Their throughput is enough to connect two PGM. Any modern notebook or ultrabook with an expanded volume of the operating memory and connected through USB 3.0 to the external HDD or SSD is capable of performing signal processing for PGM.

PGM detection subsystem with Ion 318 chip generates data with the speed of 220 Mb/s and it is enough to have gigabyte interface GigE in order to transmit information to the computer. Ion PI produces almost 8 Gb/s and this parameter in Ion PII will double. So, the detection subsystem in Proton sequencer is embedded in a powerful internal computer /2/ and the already processed information, the size of which is significantly less, is transmitted to the server. This design enables to handle information flows but at the utmost of the device's capacities. So, to enlarge the throughput of semiconductor sequencer from 10 to 100 billions bp, it will be required to use the most advanced achievements in the computer industry.

An alternative system may use more stable signals of DNA synthesis instead of the present technology designed on the basis of detection of fast diffusing protons. These signals could be the release of pyrophosphate /27/ or increase of the charge of the sequenced DNA /40/.



Conclusion

The market of new technologies of DNA sequencing is comparatively small and almost on the whole depends on budgetary funds allocated for research investigations. Its attractiveness for the investors is associated with the plausible in the nearest years explosive growth, which should be initiated by the decrease of the cost for clinical sequencing making human genome sequencing more affordable. In view of generally depleted potential for fluorescence-based technologies, major hopes relate to the development of a series of semiconductor technologies of DNA sequencing. Jonathan Rothberg, the founder of *Ion Torrent*, put into practice only one of possible versions of semiconductor sequencing based on usage of pH-sensing chips. Similar chips are being now developed in London at *DNA Electronics* for *Roche* planning to demonstrate own semiconductor sequencer in 2015 /41/. A team led by D. R. Cumming in Glasgow works out sensing chips with the embedded system of reagent delivery /42/. *InSilixa* in California develops high-performance CMOS biochips of its own design that are intended for DNA sequencing /43/.

In the nearest future, the interest in semiconductor sequencing may be shown by some major companies and corporations producing BSI image sensors of different types and/or by companies displaying their interest in the market of clinical diagnostics (*Sony*, *Samsung*, *Panasonic*, *Toshiba*, *Intel*, *IBM*, etc.). And Jonathan Rothberg will hardly be unemployed for a long time. Most likely, he and his new team will be the leader in genomic race. If it takes the place, “Rothberg sequencing” may become a customary term in scientific literature as “Sanger sequencing” is.

Acknowledgments

This work was supported by the Federal Target Program “Research and Development in Priority Directions of Scientific Technological Complex of Russia for 2007-2013” (GK No 14.512.11.0087). Special thanks go to Dr. A.V. Chemeris for countenance and to Dr. M.N. Romanov for editing and translating the review into English.

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