SIXTH FRAMEWORK PROGRAMME

SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT

Publishable Final Activity Report (Summary)
GaNano: 01.01.04 – 31.12.06

Project acronym:	**GANANO**
Project full title:	**"New Generation of GaN-based sensor arrays for nano- and pico-fluidic systems for fast and reliable biomedical testing"**
Contract no.:	NMP4-CT-2003-505641
Project Officer:	Dr. Martin Schmidt

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Europe turns to GaN for medical sensor arrays (GaNano)

Hospitals and medical laboratories have to analyze thousands of tiny samples on a daily basis. This is ideally carried out quickly, efficiency and with a high degree of sensitivity, enabling early detection of conditions such as AIDS, Creutzfeldt - Jakob disease and cancer. Rapid diagnosis can save lives, improve a patient's quality of life and also reduce medical treatment costs.

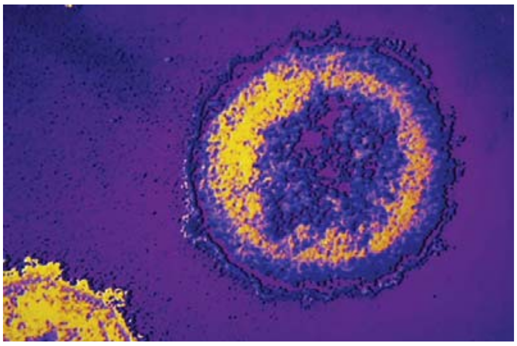


Fig.1: *GaNano is the name of an European consortium that is building a medical GaN-based tool for the detection of diseases such as cancer, AIDS and Creuzfeld-Jakob Disease.*

Fig.2: *At the heart of the GaNano instrument is an arrangement of seven labelled sensors on a translation stage that are able to analyse droplets with a volume of 50 pl to 1 nl.*

GaNano is three-year €2.3 million EU-project coordinated by the Technical University Ilmenau with eight other European partners to construct the first instrument based on GaN devices to address this need. GaN and its related alloys are well-suited to this task, and unlike the GaAs and InP families they can form the basis chemical sensors that are not degraded by various samples, including those that are acidic and alkaline. The following results and deliverables are achieved by the members of the consortium and the effect of an excellent cooperation.

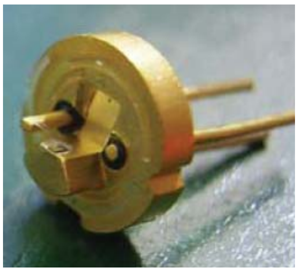
These sensors, which are the key part of the instrument, analyze the chemical properties of nanolitre and picolitre samples that are brought into contact with the device's surface. The devices - essentially transistors with an eliminated top gate or a modified active gate area - can detect dipoles, polar liquids, changes in ion concentration, and cell activity, because this alters the device's surface potential and leads to a change in current flowing through the underlying two-dimensional electron gas (2DEG).

Fig.3: *Droplets are applied to the sensors with a silicon device that uses microchannels and pumps driven by piezoelectric actuators.*

We can tailor our sensors so that they only respond to a particular type of substance by applying specific membranes to the gate area that are made from either biological cells or artificial materials. For example, members of the University of Crete and the Foundation of Research and Technology Hellas have modified our detectors to be sensitive to potassium by covering the gate region with a polyvinyl chloride membrane, which is doped with valinomycin, a large molecule made from amino acids.

Applying different types of organic membrane to our devices has allowed us to produce sensors that are selective to other important cations such as ammonium, and sodium, and also to anions such as nitrate and chloride. In each case the signal from the sensor is caused by the potential difference at the interface between the membrane and the aqueous solution that is created by the reaction between the ion carrier and the analyte ion. Experiments at the Technical University of Munich have shown that immobilized enzymes such as penicillinase can be applied to the sensor's surface, which alter the device's selectivity and sensitivity.

Our work with cations, anions and enzymes illustrates the great potential of GaN-based sensors. Detection with these devices can be extended to different biological systems by adapting the surface and the sensor, enabling a series of different sensors to be fabricated that form the basis for a multifunctional sensor array.



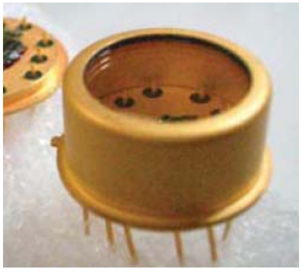


Fig.4: *The GaNano system features GaN-based lasers (left) and detectors (right).*

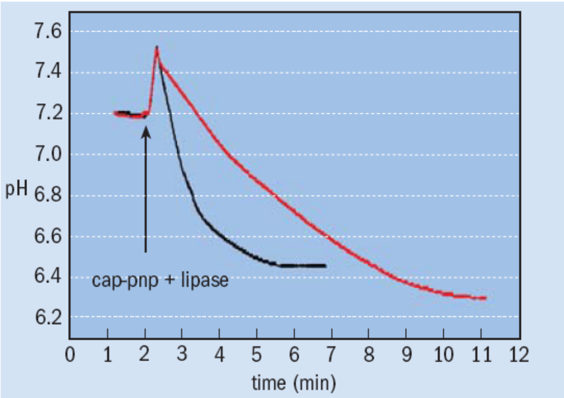
Manipulating the droplets

We apply our tiny samples to the sensor's surface with a dispenser that is similar in design to the head of an inkjet printer. Forcing a solution through a membrane and orifice creates a liquid jet that can be controlled to deliver a sequence of droplets at rates of up to 300 per second onto a moving sensor array. One or more droplets are applied to each sensor, and by adjusting this system droplets can be delivered at speeds of 1-3 m/s and volumes of 50 picolitres to 1 nanolitre.

Our project also benefits from GaN's optical transparency at shorter wavelengths, where silicon, GaAs, and InP are highly absorbing. This transparency enables biological samples to be identified by their fluorescence spectra, which often contain prominent features at ultra-violet wavelengths.

Members of the Madrid Polytechnic University, TopGaN, and Unipress have developed various GaN-based devices for this type of measurement, such as lasers and photodetectors. For determining the concentration of lipase - a key enzyme in the human body that is required for the absorption and digestion of nutrients in the intestines - we have built laser diodes emitting at 412 nm and 419 nm, and photodetectors that contain an InGaN-based filter that restricts detection to the 400-415 nm band, which negates the need for an external dielectric filter. These devices could be used to see if a patient has a high concentration of human pancreatic lipase, which could be caused by gallstones or excessive alcohol consumption that led to pancreas inflammation.

All of our GaN devices have been installed in a temperature-and-humidity-controlled glove-box to reduce the evaporation rate of our various samples. The fast evaporation rate is a consequence of our "open" design, but this approach does benefit from fast mixing between solutions. Systems with a "closed" design, which route liquids through micron-sized channels, are more common. Although they do not suffer from fast evaporation rates, mixing between solutions occurs very slowly.



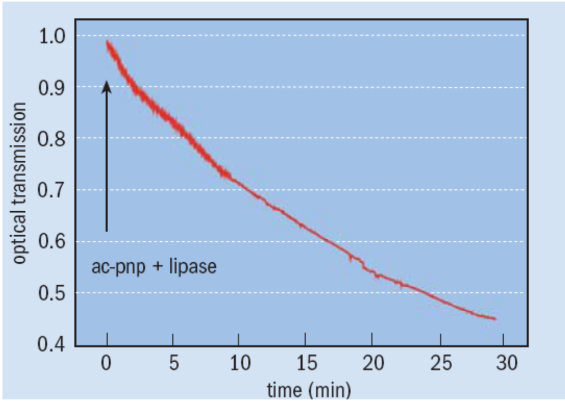


Fig.5: *The AlGaN/GaN sensor can monitor the change in pH as the enzyme lipase drives the decomposition of 4-nitrophenylcaprilate into capryl acid and 4-nitrophenol. Dilution of the enzyme reduces the rate of change of the pH value (left). GaNano's transparent sensor is compatible with absorption experiments that employ GaN lasers and detectors. The absorption data reveals the concentration of the dye 4-nitrophenol, which is product of the decomposition of 4-nitrophenylcaprilate (right).*

Making novel measurements

At the Technical University of Ilmenau first continuous optical and electrical and measurements with the GaNano system were carried out on lipase, which provided the catalyst for the decomposition of organic molecules (either 4-nitrophenylcaprilate or 4-nitrophenylacetate) into an acid and a dye (4-nitrophenol). Our electrical measurements, which used AlGaN/GaN sensors that did not require any modification to provide a selective response, monitored the change in pH of the products of this reaction. After adding lipase the system's pH initially increases from 7.2, and then decreasing as expected to 6.4. The metabolic rate, which is the rate of change occurring in the enzyme, can be calculated from the pH value and a titration of the resulting acid. The optical measurements determined the concentration of the other products of the reaction, the dye, by measuring the transmission characteristics of the solution.

Our electrical and optical measurements have allowed us to track the behaviour of both products produced in the reaction, and observe the influence of lipase. Together with our industrial partners Analytic Jena and the European Aeronautic Defense and Space Company the next step is to integrate these components together to produce an instrument that can simultaneously monitor both of the products created in this reaction. Integrating these components demands a high degree of precision, because droplets with volumes of just 50 picolitres have typical diameters of 60 µm. We are now addressing this challenge, and when this is complete we will begin to make measurements on other biological materials. This will ultimately lead to the construction of a GaN tool for rapid identification of various diseases.

Exploitation Plan

The economic impact of the project is two-fold. First is associated with the potential economic benefits that will result from direct exploitation of project results. Second, indirect, is associated with cost savings resulting from the potential exploitation of project results. The potential economic benefits from potential exploitation/commercialisation of project results include the sales of the end-product (integrated system), but also projected sales of intermediate results (individual components). The sales of the multifunctional lab-on-a-chip

system can initially range between 100-200M EUR, assuming 3-5% market penetration (with a 10-20% operating profit margin) of the 2-3 billion EUR (conservative estimate for 2006-2007) market for miniaturized solutions using Microfluidics/Microchip technologies within life sciences. EADS (and Analytik Jena), a company which was integrated into the GaNano consortium to replace the former partner General Electrics (GE) is actively involved with this technology, and will work with this team to bring the *multifunctional integrated system* and *the new generation of GaN-based sensor arrays for nano- and pico-fluidic systems for fast and reliable biomedical testing* to market in a timely manner as successes are achieved in the various stages of the research. EADS, TopGaN and AJ realized the integrated sensor system to identify coli bacteria in water with extreme sensitivity. GaN-based nanotechnology developed within the project will be used to realize products which enable the detection of single bacteria by fluorescence spectroscopy.
The production cost of the proposed multifunctional integrated system immediately after the stage of research and development can be estimated by the production cost of the subsystems, and in the initial stages will range between 50.000 – 80.000 EUR. Development of the time consuming technology and the manpower needed to build and test the integrated system dominate the expected cost. This cost is expected to drop by about a factor of 2-3 to 20.000 – 35.000 EUR once the production capacity exceeds 1.000 systems per year. Target users of the integrated system are hospitals, medical test laboratories, pharmaceutical producing companies and scientific institutions. After the spin-up period sale volumes of about 1.000-5.000 in Europe and 10.000-20.000 world wide are expected. Such sales would generate a turnover in the range of 250-500 MEUR. Future developments of the integrated system into a cheaper compact and portable device focusing on selected capabilities (e.g., bacteria detection, water impurity testing, etc.) would target the market of individual end users such as physicians, various portable water supply systems, etc. Such development could increase sales volumes to over 100.000 units.

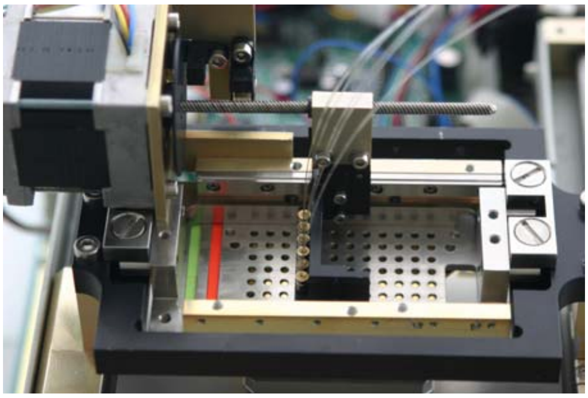


***Fig.6**: Fiberoptic Scanner used in the real-time PCR device of AJ, based on the "SpeedCycler" – the high speed thermocycler of AJ*

The indirect economic impact results in potential cost savings to be realized via reduction of medical cost treatment due to early detection of certain disease associated proteins and pathogens, shorter time-to-market for new pharmaceuticals, and higher efficiency of sampling of large numbers of very small samples (nano- and pico-droplets). Finally, additional economic benefits are generated through application of the smaller, cheaper, and portable versions of the system for faster, more efficient, and statistically more reliable sampling of water quality in commercial aircraft as well as participation of large industrial partners (AJ and EADS) significantly increases the potential for efficient commercial exploitation of project results. With already well-established commercial partners, high-capacity production facilities, and distribution systems, these partners can assure shorter time to market and broader market reach for the commercialised products.

Analytik Jena immediately benefits from the technology and the selection of appropriate materials for the production of the fiber optic sensor head. Another application of such fiber optics can be found in an instrument for genetic analysis which is currently under development at AJ. This so called “real-time PCR – thermocycler” will presumably be launched to the market in 2007. The setup of this instrument was filed as a patent application in March 2006. A central part in that application is the use of fiber optics with very low auto-fluorescence. Producing such fibers required screening of a variety of fibers, different fiber buffers and adhesives which was done during the GaNano project in order to create the fiber optic sensor head. Fig.6 shows the fiberoptic scanner which is part of the fluorescence detection system of the thermocycler used for amplification of genetic material.
Another application of results of the GaNano project can be found in a new device capable of reading absorption and emission spectra from nanoliters of liquids which is currently under development at AJ. The instrument employs a variety of microchannels on a chip in which the samples can be filled. The wavelength range of the absorption and fluorescence spectrum measured, 230 nm through 1000 nm, is similar to a cuvette spectrophotometer. The spectrum is recorded simultaneously by a photodiode array or a CCD matrix. The spectrally resolving detector is the same as in the GaNano project but employs a different grating. In order to guide light from the light source to the microchannel and from there to the detector we employ optical fibers.

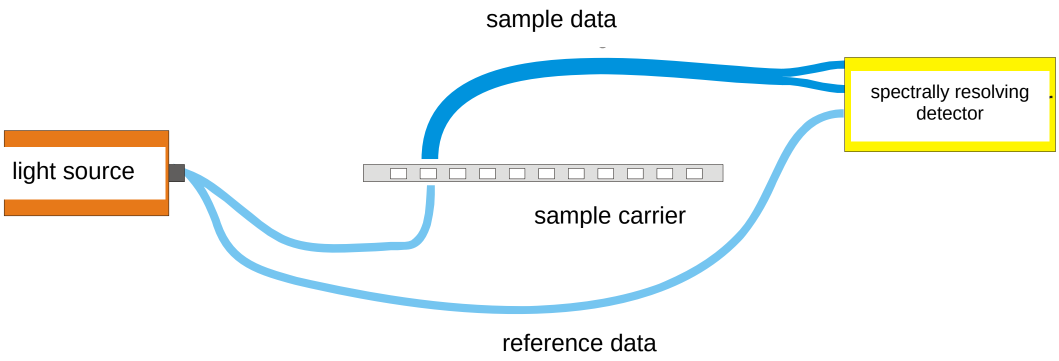


Fig.7*: Scheme of the nanoliter spectrophotometer*

Using a grating with imaging quality one can establish a multi channel detector capable of measuring multiple samples and references at the same time. Such a setup is useful for high throughput detection and furthermore eliminates the error which occurs if sample and reference are measured sequentially.

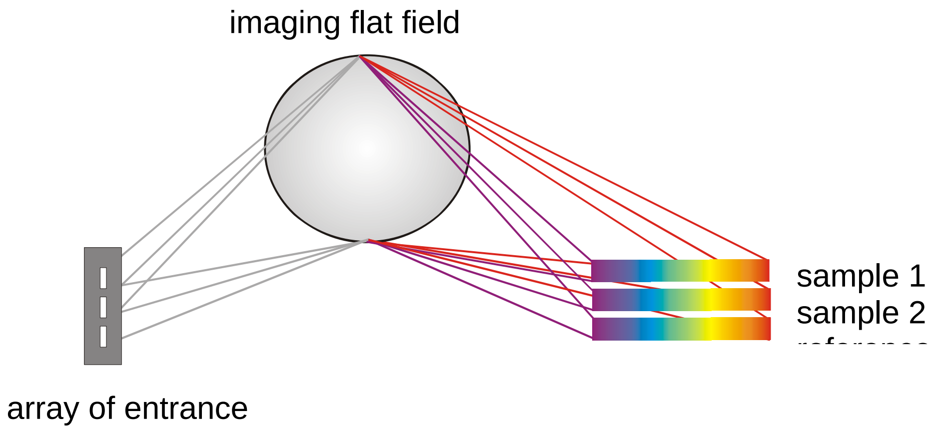


Fig.8*: Scheme of a multi-channel spectrophotometer*

Since 2005 Analytik Jena is also engaged in the field of liquid handling. The first commercial product of AJ in this field is the pipetting robot “FasTrans” which allows for flexible pipetting in the volume range 0.5µl to 30µl using traditional positive displacement techniques. The device is used for the preparation of microplates for ELISA and PCR assays and can be equipped with different pipetting heads having 1, 3 or 6 pipetting channels. In the future AJ plans to extend the volume range to the nanoliter scale which is only possible by using piezoelectric dispensers. Such elements are produced by the contractor TUI and are part of the integrated system. In a future projects between AJ and TUI this dispensing technology could be the basis of an ongoing cooperation.

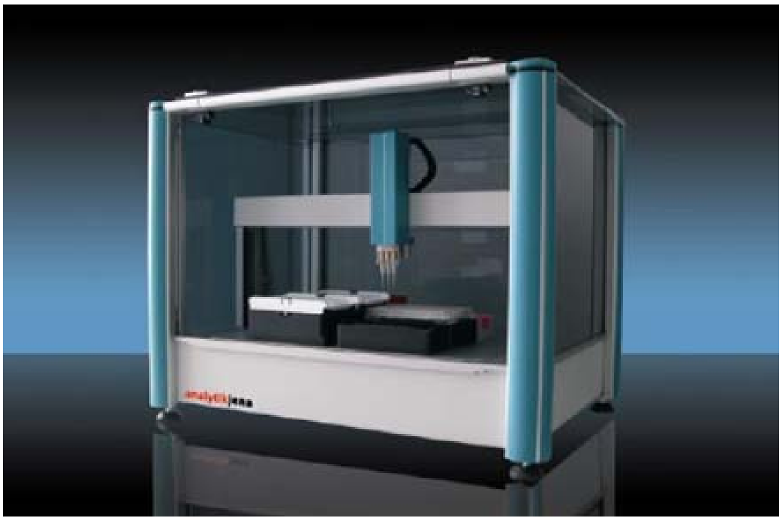


Fig.9*: The pipetting robot “FasTrans” – a flexible tool for liquid handling*

Outside of the biomedical sector, the multifunctional system has potential applications in the environmental sector. While no market research data have been compiled additional revenue could clearly be generated in, for instance, the area of water quality sampling. One possible application is testing of on-board water supply in aircraft prior to their release for operations. Currently this process is very complicated and time consuming. Implementation of the lab-on-a-chip system potentially represents significant cost savings resulting from faster commissioning of aircraft into service. The new approach of GaN based integrated optical and electronic sensor technology and measurement methods also offers possibility for onboard water quality control reducing health risks associated with the microbiologically contaminated water. Broader water quality related applications can be foreseen provided the production costs of a simplified system can decrease considerably (by a factor of 3-5). Such potential applications include trains, boats, ships, swimming pools, etc. Furthermore the system can be applied to water quality instrumentation installed for security purposes and for wider monitoring of water quality in distribution by the utilities. Results of the GaNano project related to lab-on-chip systems for drinking water control will be further used, developed and exploited towards market by EADS.

Furthermore, the benefits from potential sales of the end-product components (blue and UV LDs of the specified parameters) can range from 10 to 20M EUR (based on market research and predictions of Strategies Unlimited, Optech Consulting, and TopGaN estimates). The UV-emitters production plans of TopGaN (SME) go far beyond the applications in the pico-liquid integrated systems. During GaNano project execution, TopGaN, a start-up from the Institute of Physics Polish Academy of Sciences, has developed its first commercial products (violet laser diodes) that are now being sold as engineering samples (around 50 in 2006).

They were purchased almost entirely for constructing instruments used in medicine, biology and environmental protection (topics covered by GaNano project). Most of these instruments are still in a development stage, therefore, TopGaN is being invited to be a partner for both, commercial ventures with European companies, as well as in the new European projects prepared for the Framework VII.

The most promising field of TopGaN lasers application is detection of nitrogen oxides (NO_x), gases emitted by ill humans and animals, rotting fruits and vegetables, many explosive materials as well by polluting wastes and manufacturers. The NO_x sensors were developed by the Technical Military University (WAT) in Poland using the light source (laser diode) developed within GaNano project. It is expected that the commercialization of the sensors will be at the end of 2007. The detection limit of NO_x will be of a fraction of ppb, more than an order of magnitude lower than using much more expensive and heavier instruments. It is worth to mention that TopGaN laser diodes for this application (pulsed mode operation with ultra high power during the pulse) have the best-world parameters.

The success in technology development, using the laboratory equipment, as well as the market interest led the investors to a decision of equipping TopGaN with the advanced production equipment. After the 9 mil Euro investment completed at the end of 2007, TopGaN is supposed to produce in 3-4 years 20-40 thousand laser diodes per year mainly for the markets of sensors in medicine, biology and environmental protection. For GaNano project execution, TopGaN employed 6 scientists. After the project, they will keep their positions.

In summary, the technological advances and the prospective exploitation areas of the project results achieved in the GaNano-project are likely to increase European competitiveness in high-technology areas of optoelectronic integrated systems for bio-medical and environmental applications.