



NovaChip Evanescent Resonator Technology

The NovaChip microarray platform exploits a physical (optical) amplification scheme to enhance signal intensities – enabling gene expression from minute samples containing less than one nanogram of RNA.



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Dieter Neuschäfer graduated from the University of Göttingen (Germany) with a degree in Physics, and completed his PhD at the Max-Planck-Institute in Göttingen. After spending a post-doctoral research period at the University of Colorado (Boulder, Co), in 1987 he joined Ciba-Geigy's Central Research Laboratories (Basel, Switzerland). During the 1990s he developed, amongst other things, planar waveguide biosensors for Ciba-Geigy's Diagnostic Division and the Evanescent Resonator microarray platform for Novartis. Since 2002, he has been Head of Technology Development within the BioMarker Development Department of Novartis Pharma (Basel, Switzerland).



Estelle Marrer earned her PhD in 2002 under the co-guidance of Professor Malcolm GP Page at Hoffmann La-Roche and Professor Frank Pattus at the Université Louis Pasteur of Strasbourg. She then spent two years as a postdoctoral fellow at Basilea Pharmaceutica, a biotechnology company focusing on the discovery and development of novel drugs in the fields of anti-bacterials, anti-fungals and dermatology. In 2004, Dr Marrer joined Novartis Pharma AG (Basel, Switzerland), where she is currently a gene expression analyst in the Department of Biomarker Development.



Wolfgang Budach received his PhD in Chemistry from the University of Göttingen/Max-Planck-Institute for Biophysical Chemistry (Germany) in 1992; during his studies, he specialised in biophysics and fluorescence techniques. He was Co-founder and CEO of a start-up company for scientific instruments, and Postdoctoral Fellow in Paris at the Centre d'Etudes des Saclay. Since 1995, Dr Budach has been developing new technologies for Novartis (Basel, Switzerland), and is author and co-author of various patents and publications dealing with evanescent field-based sensors.

Sequencing the human genome and other model organisms has generated huge databases with millions of DNA sequences. We know that there are approximately 30,000 genes in the human genome, and hundreds of thousands of proteins that code for biological information. In combination with analytical technologies, such as DNA, oligonucleotide and protein microarrays, this knowledge allows research on gene regulation, function and pathways.

FLUORESCENCE-BASED MICROARRAY PLATFORMS

To speed up drug development, powerful miniaturised, multiplexed and automated tools have been developed for the large-scale use of genomic DNA microarrays. Microarray chips contain vast numbers of capture probes with known identity/sequence on a flat substrate allowing massively parallel gene expression studies. Capture probes are immobilised on the substrate by photolithographical means, by ink-jet deposition or by contact printing. Labelling of the study material with

fluorophore molecules allows quantification of gene abundance by scanning the whole chip with a focused laser beam. Changes in fluorescence intensities between individual samples indicate differences in gene expression, and provide valuable information about all kinds of biomarkers (for example, disease-, safety-, efficacy- and mechanism of action-related biomarkers).

A major drawback of today's microarray applications is the relatively large amount of RNA required for gene expression profiling. The market leader, Affymetrix (Santa Clara, CA) recommends profiling with 5µg for the standard GeneChip® protocol. However, a large portion of samples to be analysed contain considerably lower amounts of RNA. Additional rounds of RNA amplification are an alternative, but will lead to reduced fidelity of correlation coefficients (1).

NOVACHIPS

The Novartis Evanescent Resonance platform (NovaChips) approaches an alternative route. Instead of



amplifying the relevant biological material prior to hybridisation, NovaChips exploit a physical (optical) amplification scheme to enhance signal intensities. At resonance condition, the fluorophore labels attached to the samples are excited much more efficiently compared with a conventional microarray platform, leading to increased fluorescence signals and improved limits of detection, thereby lifting low expressed genes above background levels.

To boost fluorescence yield, various amplification schemes have been developed in the past. The most sensitive devices are those based on evanescent wave excitation. They make use of coherent light that is trapped in and confined to an extremely thin surface layer with an average thickness of 100-200nm, smaller than the wavelength of light. Energy confinement creates strong electromagnetic evanescent fields extending for a small distance outside the chip – typically 1 micron. Examples of evanescent wave excitation schemes are planar waveguide sensors (2), surface plasmon resonance devices (3) and resonant mirrors.

A disadvantage of all the above-mentioned schemes is the need to carefully align the devices relative to the excitation light beam due to the nature of the underlying resonance phenomena. As a consequence, these devices are not compatible with available microarray-related products and services. NovaChips, on the other hand, overcome such limitations without sacrificing their superior sensitivity.

DESIGN CONSIDERATIONS

The NovaChip microarray platform consists of a glass substrate with a sub-micron optical grating/diffractive element etched into it, and a thin dielectric layer of high refractive index material coated on top of the corrugated surface. The diffractive structure expands over the entire slide and is preserved after the coating step. The high degree of sensitivity of the NovaChip is based on an interference phenomenon between the incoming zeroth- and the diffracted first-order waves, as shown in Figure 1. The effect is called abnormal reflection (4). At resonance position – that is, the angle where the abnormal reflection occurs – part of the incident light is coupled into the dielectric high-refractive index layer. By choosing the appropriate angular condition, a destructive interference is created in the direction of the transmitted beam leading to almost total reflection of the incident light. The

Figure 1: The effect of abnormal reflection stems from interferences in the corrugated layer system between the incident laser light and the diffracted first-order waves. By choosing the appropriate angular condition, a destructive interference is created in the substrate leading to almost total reflection of the incident light. The effect occurs with light incident either from the cover or from the substrate side, and can be shifted to normal incidence.

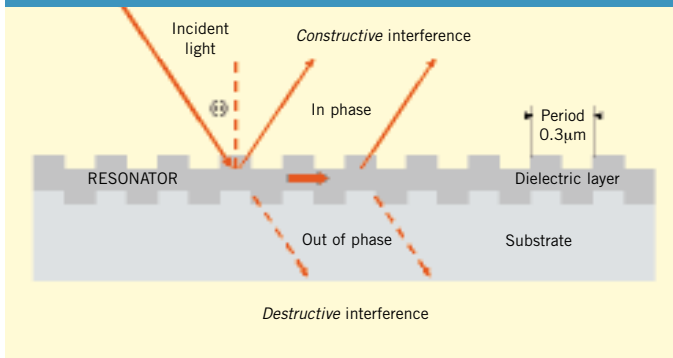
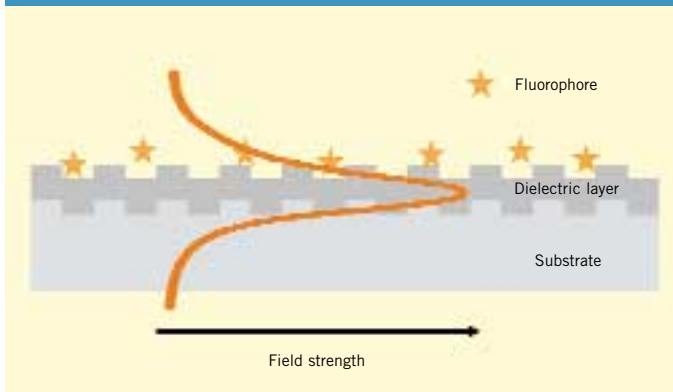


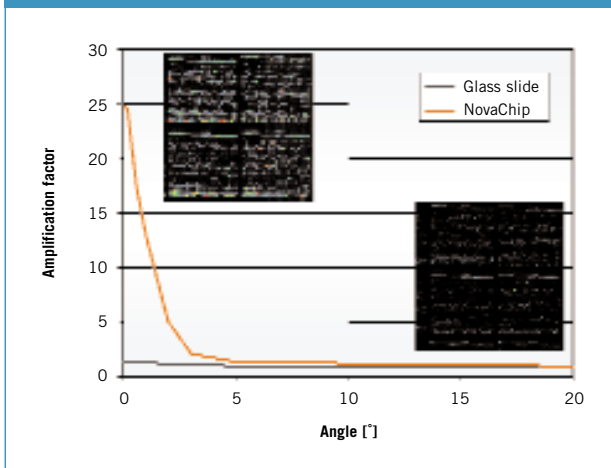
Figure 2: Cross-section of the NovaChip platform. At resonance condition, confinement of the excitation light inside the high refractive index layer creates intense evanescent fields (orange curve) leaking both into the substrate and into the buffer layer. Field strengths decay exponentially to zero within typically 1-2 microns.



resonance angle is defined by the effective refractive index, and the ratio of grating period and laser wavelength (5).

Associated with this abnormal reflection condition is the formation of intense evanescent fields at the chip surface due to energy confinement in the thin dielectric layer (typical thickness, 100-200nm, see Figure 2). The electromagnetic field strengths at the surface of such a platform might exceed the fields of the free-flowing laser by four orders of magnitude. As a consequence, the fluorophore labels in the close vicinity of the chip surface are excited much more efficiently compared with conventional microarray platforms, leading to larger signal intensities. Dye molecules that are not attached directly to the surface are not affected, due to the exponential decrease of the evanescent fields (see Figure 2).

Figure 3: Demonstration of the amplification potential of NovaChips using a commercially available laser scanner (Tecan 200). At resonance position (normal incidence), the overall signal intensities are increased by a factor of 25 compared with off-resonance (orange curve). Signal intensities of a glass chip (grey) are not affected by adjusting the angle of the incident light between 0° and 20°. The two inserts show the actual fluorescence images of the laser scanner at 0° and 20°, respectively.



A very important parameter for consideration is the groove depth of the diffractive structure. By etching grooves of 40nm and deeper into the platform, the resonance width for abnormal reflections is well above 1° for the given design, allowing alignment-free handling of the NovaChips. In addition, for reasons of practicability, we have shifted the resonance position to normal incidence by choosing the appropriate thickness of the dielectric layer (6). This attribute allows the NovaChips to be compatible with commercially available instruments like laser scanners, CCD-based detectors and confocal microscopes. The application examples given below are derived from scanner experiments.

To demonstrate the amplification potential of the NovaChips, we used a Tecan LS 200 scanner. Whereas most laser scanners allow no tuning of the optical properties of the laser beam, the Tecan LS 200 (7) enables the user to freely choose the angle between chip surface and incoming laser beam. With a standard microarray slide made out of glass or plastic, this procedure does not affect the signal (see Figure 3, grey line). However, by approaching the resonance position of a NovaChip platform at normal incidence, the fluorescence yield increases by at least one order of magnitude. The two inserts show part of the actual fluorescence images of the laser scanner at 0° and 20° respectively. It is obvious that other scanners with fixed normal incidence geometry will exploit the amplification potential of the NovaChip platform as well. For deviant

geometries, laser wavelengths or dye labels, the resonance position can be adjusted to any given angle.

TECHNICAL ASPECTS

NovaChips have been developed to be compatible with commercial equipment on the market – switching from self-spotted 1" x 3" membrane, plastic or glass slides to 1" x 3" NovaChips requires no additional hardware. We are able to spot 70mer oligonucleotides as capture elements by contact-printing onto the chips. The spot diameter is 100-150µm. All samples are labelled with one dye (Cy5 fluorophores). Control and treated samples are hybridised onto two different chips. Read-out is by conventional laser scanner (Tecan LS 200, Agilent Technologies or Packard ScanArray 4000).

Since the biological material available for microarrays is often very limited, RNA extraction protocols and RNA quality obtained from minute samples are of paramount importance. Therefore, different parameters have been optimised to recover a high RNA yield at best quality. Other protocols like Reverse Transcription, *In Vitro*-Transcription (IVT) or PCR amplification have been developed to amplify RNA isolated from limited amounts of tissues or cells. With NovaChips, the standard protocol (without amplification!) can be used for samples containing only 10ng of total RNA. Below this limit, the NuGen protocol provides a linear amplification alternative with no loss in data fidelity. Due to the (physical) NovaChip fluorescence enhancement by evanescent field excitation, no additional biochemical amplification steps are necessary. This avoids any bias in correlation coefficients as mentioned earlier.

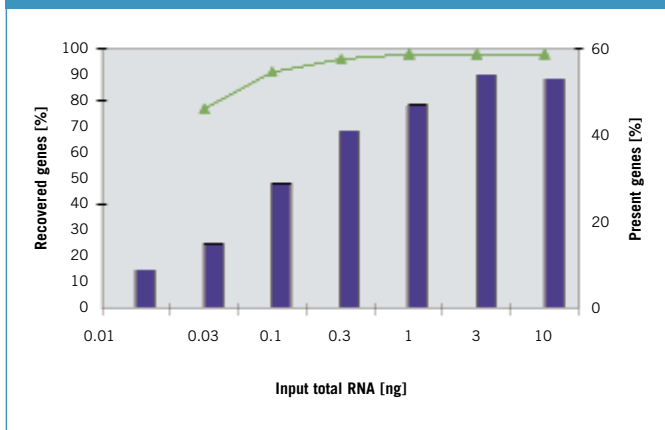
APPLICATIONS IN DRUG DEVELOPMENT

NovaChip technology renders possible the entire genome profiling of minute samples, whereas any other microarray technology would require more starting material. This is one of the main areas of interest at Novartis. To demonstrate the performance of NovaChips in terms of sensitivity and small sample size, we carried out a dilution and correlation study.

Extracting RNA from rat brain scrapes, we started with 10ng of total RNA and reduced it by factors of three down to the pg level. The labelled cDNA (NuGEN protocol) was then hybridised to NovaChips. In Figure 4 (page 82), the bars represent the percentage of genes

above background noise (right scale). Above 1ng of total RNA input, we measure a constant level of genes present with excellent correlation between the individual concentrations. Below 1ng of RNA, the number present starts to diminish. Confidence values between two technical replicates are still above 0.95. Analysing the data shows that 97% of the genes being detected with only 10pg (!) of total RNA are also present at 10ng (the line marked with triangles in Figure 4). In other words, performing gene expression analysis with NovaChips using samples with a total RNA input in the pg range without additional RNA amplification is possible, and will introduce only minor bias to the expression profiles.

Figure 4: Dilution and correlation curve demonstrating the performance of NovaChips at low RNA yields. The bars show the percentage of genes above background in a hybridisation experiment starting from RNA amounts between 10ng and 10pg (rat brain tissue, Operon rat genome set version 1.1). At 100pg total RNA, still 45% of genes are called present. The green triangles represent the percentage of all the genes being detected at 10pg starting material, which are also marked present at the corresponding measurements with larger RNA yield.



This opens up new possibilities for profiling minute samples from all kinds of biopsies, body fluids and single cells previously not accessible by GeneChip analysis. Combined with sampling by Laser Capture Microdissection (8), NovaChips will deliver a clearer expression profile than with homogenised material.

CONCLUSION

A new, sensitive and easy-to-use transducer for the analysis of all kinds of biomolecular affinity systems has been developed and validated. The NovaChip platform combines superior sensitivity with exceptional reproducibility and ease-of-use. The device exhibits a uniformly, nano-structured surface giving rise to local energy confinement of the incident light in a very thin dielectric metal-oxide layer of sub-micron dimensions.

When compared with conventional glass slides, the Evanescent Resonator enhances the fluorescence yield of surface-bound fluorophores by at least one order of magnitude. Combined with state-of-the-art RNA extraction and labelling protocols, NovaChips allow gene expression from minute samples containing less than 1ng of RNA without additional amplification. The chips are compatible with most commercially available laser scanners, and confocal microscopes, as well as portable or miniaturised CCD read-out equipment.

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Note: NovaChips are not commercially available from Novartis, but the company will negotiate licensing of the NovaChip Evanescent Resonator technology with interested parties in the microarray field.

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