Point Mutations of DNA Sequences

Michaël CANVA - *Laboratoire Charles Fabry, Institut d'Optique, CNRS, UMR 8501, Bat 503, Centre scientifique, 91403 Orsay, France*

Genes can be mutated in several different ways. The simplest type of mutation involves a change in a single base along the sequence of a particular gene (much like a typographical error in a word that has been misspelled). In other instances, one or more bases may be added or deleted. Sometimes too, segments of a DNA molecule are accidentally repeated, deleted or moved.
DNA mutations generate genetic disorders that are responsible for hereditary diseases and have a predominant role in many diseases such as cancers.
Using GenOptics SPRi sensitive technology (Surface Plasmon Resonance imaging) a method was developed to detect DNA point mutations.

Experiment

The gold sensor surface (the glass prism) was covered with a mixed self-assembled multilayer based on electrostatic interactions made of 3 layers of MUA: 11 Mercapto-undecanoic acid, PEI: poly(ethyleimine) and ExtrAvidin. Matrices composed of 100 or 196 spots (10 x 10 or 14 x 14 matrices) were prepared and data from all parallel hybridisation kinetics was collected.

As this technique is highly sensitive and very precise it enables accurate identification of mutations even at a single base mismatch in an oligonucleotide sequence.

In addition, the recognition specificity of functionalized spots is such that each spot reacts only with the relevant complementary sequence within a mixture of different sequences. For example, by reconstituting patients genotypes with appropriate oligonucleotide mixtures (homo or heterozygote), it is possible to determine their genotype with no ambiguity.

The results of the experiments are published in Sensors and Actuators, 2003, B94, 313-323.

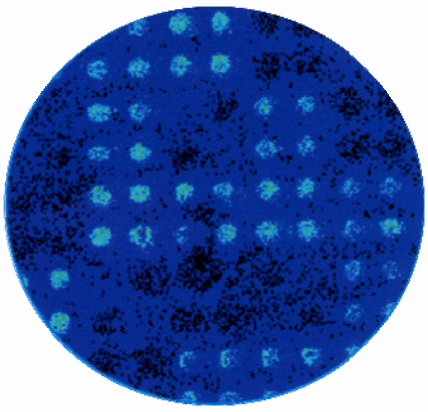

Image a: Image of the gold surface bearing 100 spots distributed on an area of 16 mm², having reacted with an oligonucleotide mixture reproducing the conditions of a patient genotype.

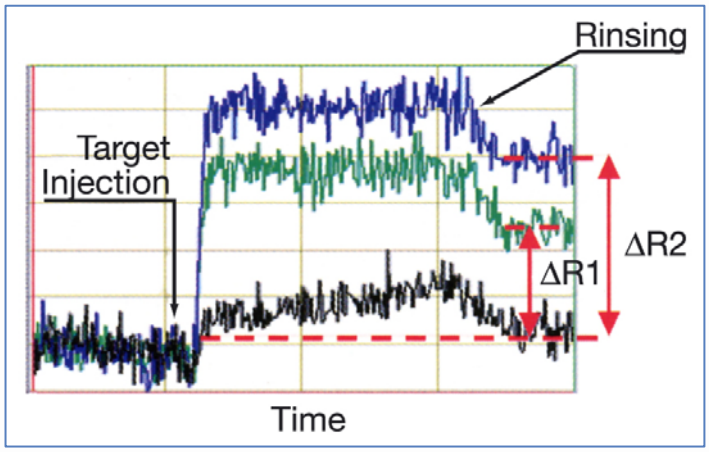

Fig. 1: From the image we observe kinetics associated with spots reacting respectively to a complementary sequence (ΔR2), presenting a mutation (ΔR1) or non relevant (ΔR=0).

The system makes it possible to monitor simultaneously several hundred measurements, and additionally, directly compare the effects induced by the variation of one or several parameters. An example is given in Figure 2, showing the variations of an hybridisation signal between probe and target of various oligonucleotide lengths.

Only one gene microtiter plate was prepared with different genotypic sequences grouped in pairs of various length presenting pairwise mutations (either by mutation or deletion). A mixture of target oligonucleotides including one of the two (wild or mutated) species, corresponding to each of the pairs was injected in the detection cell.

Using this technique, it is possible to quantify in a single run all kinetics corresponding to an entire column of the sensor matrix, and this for each sequence studied.

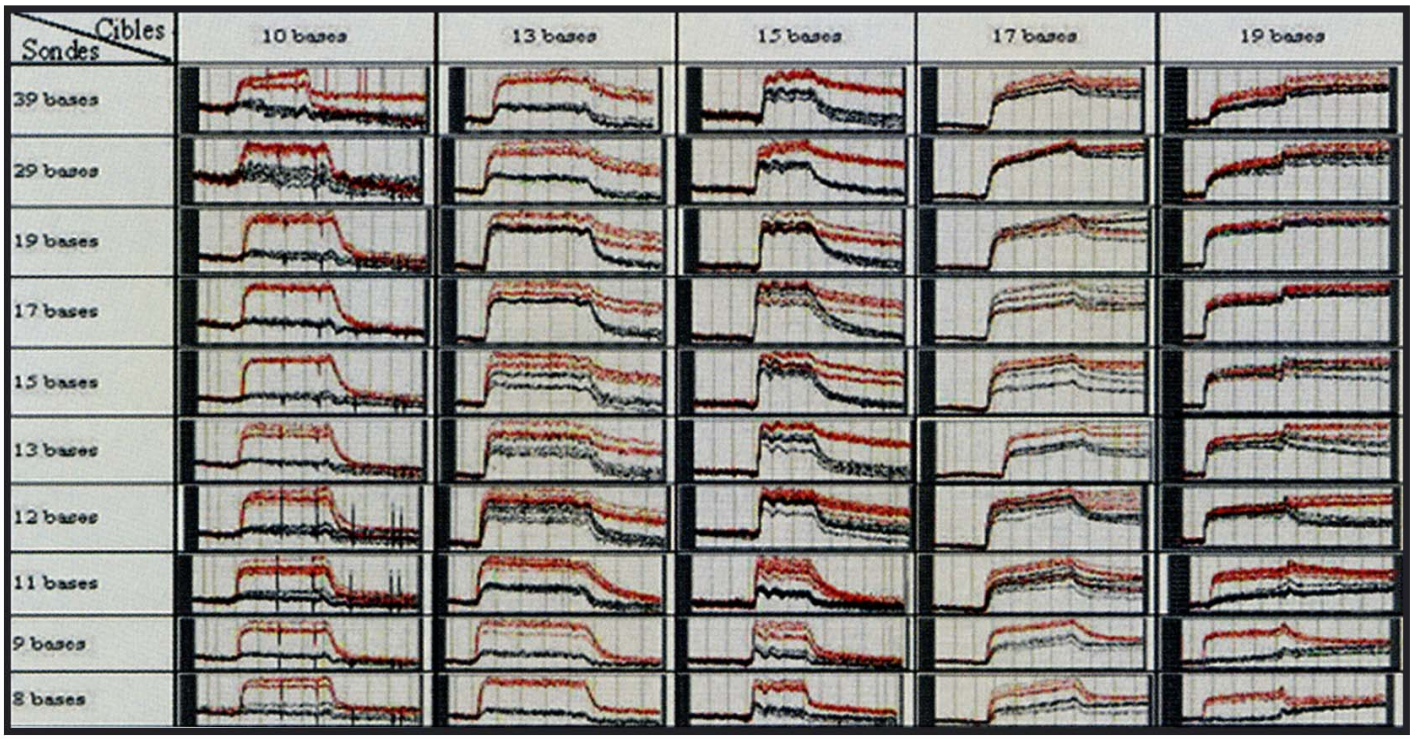

Fig. 2: Hybridisation kinetics recorded during 20 minutes periods. Rows show targets of various lengths

Figure 2 clearly demonstrates that reflectivity variations are substantially higher for the signals corresponding to totally complementary strand hybridisation than they are for the signals corresponding to a mismatch, it can also be noticed that signals are much more differentiated when one of the sequences, either target or probe, is of short length.

On the contrary it becomes more difficult to differentiate the signals when two interacting probes or targets are of a long length.

The interactions between totally complementary strands are shown in red/gray, those with partially mutated strings in black (the mutation here is one base substitution (G➞A) at the centre of the probe sequence). Interaction kinetics clearly depends on these parameters.