



 biospace lab

μ imager

Application Notes

Microarray differential screening with two radioactive probes on the μ Imager™

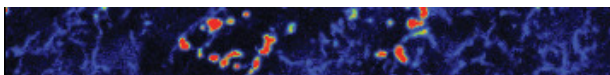
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Goal :

There are several major technological challenges to use high-density probe arrays for large-scale gene expression screenings :

- 1) the analysis of very small quantities of tissues or cell populations,
- 2) the detection of rare messenger RNAs (mRNA),
- 3) the detection of small modulations of gene expression that may be of major biological significance,
- 4) the detection and quantification, in a single sample and during the same experiment, of numerous mRNAs the amounts of which may differ by several orders of magnitude.

All these challenges involve the issue of signal-detection sensitivity. To improve this sensitivity, we have developed experimental procedures and signal filtering algorithms making radioactive labelling highly suitable for gene expression screening on microarrays. These new procedures make it possible to perform simultaneous hybridisation of two differently-labelled radioactive probes on a given microarray.



Material and methods :

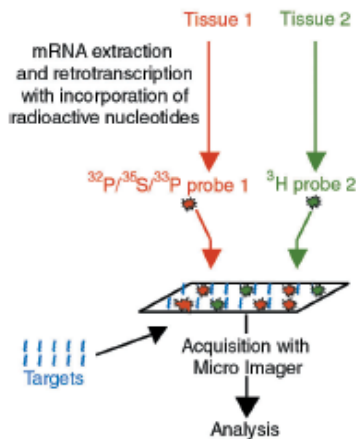


Figure 1 : mRNA was extracted from cells or tissues and reverse transcribed into single-strand cDNA probes. Probes were labelled by incorporation of radioactive nucleotides during their synthesis. The labelled probes were denatured and hybridised to the microarrays. Radioactive images were acquired with a Micro Imager.

^{33}P -dATP and ^3H -dCTP were respectively used to label two different probes synthesised from mRNAs extracted from two different tissues. These probes were simultaneously hybridised on a unique microarray. The radioactive emission resulting from the two isotopes was simultaneously acquired with the μ Imager in real time, providing a global signal. Analysis of hybridisation results was then performed using a new signal filtering algorithm, discriminating and quantifying the radioactive emissions specific to each isotope. Specific differences in gene expression between the two tissues were detected, as illustrated on fig. 2.

The μ Imager was proven to fit the spatial resolution and detection dynamic range requirements for DNA microarray analysis.

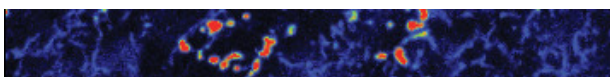
Sensitivity was tested by spotting on the microarray a 271 bp PCR product control corresponding to a weakly expressed gene (less than 1 mRNA molecule out of 500.000 mRNA molecules in total brain tissue). We were able to use as little as 100 ng of whole-brain polyA RNA for probe synthesis and still detect this mRNA, without any probe amplification. This corresponds to approximately 5 mg of starting neural tissue.

This mRNA was detected with a signal 4 fold higher than the background.

Results :

Hybridisation images were obtained with 50 ng of ^{33}P -dATP labelled probe and 50 ng of ^3H -dCTP labelled probe from two different tissue samples. After initial digital acquisition of the radioactive image, including both ^3H and ^{33}P labelling, the data were filtered to segregate the image corresponding to ^3H Beta disintegrations (the green spots of the microarray) from that corresponding to ^{33}P Beta disintegrations (the red spots), each being representative of the hybridisation result of one probe.

The yellow spots correspond to the microarray clones that were both ^3H - and ^{33}P -labelled. On the right side of the three microarray images, a spot of ^{33}P , one of a mix of ^3H - and ^{33}P and another of ^3H were set down on the arrays as controls for filter segregation of ^{33}P -beta from ^3H -beta disintegrations.



On the three graphs on the right, the respective quantitative contributions of each label to each pixel is shown along a line as an example (the arrow on the graphs points the signal intensity of the probes hybridised on the microarray spot marked by an arrow). Data are not normalized.

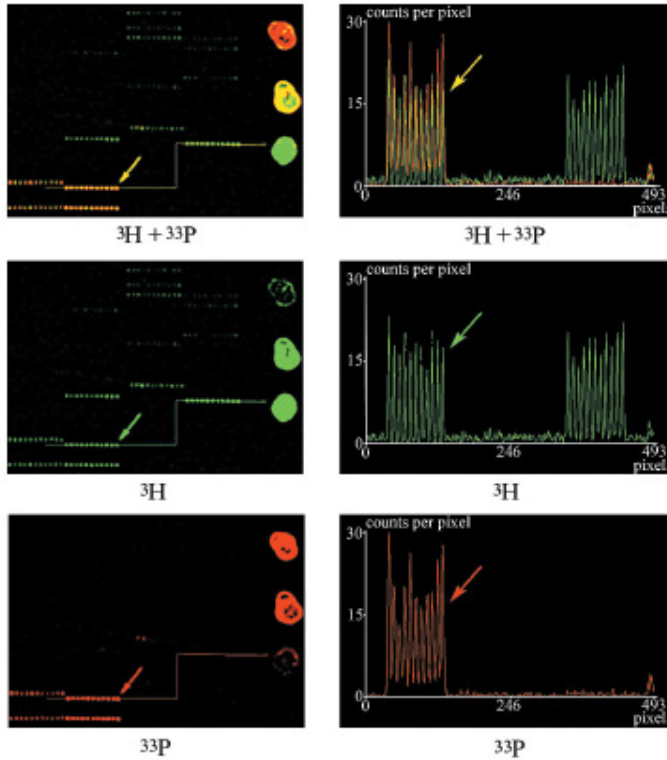


Figure 2 : 100 ng of cDNA was radioactively labelled in presence of (^{32}P) dATP or (^3H) dCTP using poly(dT) priming and purified. Probe synthesis and labelling were performed in 25 μl in the presence of 0.8 mM dGTP, dTTP, 10U AMV reverse transcriptase (Prolabo), and 50 μCi (^{32}P) dATP and 0.8 mM dCTP or 100 μCi (^3H) dCTP and 0.8 mM dATP for phosphorated or tritiated probes, respectively, by incubation of the mixtures at 42°C for 2h. The probes were added to the hybridisation buffer (3.5X SSC, 0.3X SDS), and hybridized at 60°C for 16-17h. The arrays were rinsed at room temperature in 2X SSC, 0.1%SDS, then 2X SSC, then 0.2X SSC, for two minutes each. Targets were PCR products of 300 to 1500 bp spotted on polylysine coated slides with a 250 μm centre-to-centre spot spacing.

Conclusion :

The dual label detection capability of the μ IMAGER allows differential gene expression with radioactive labels, an option up to now only possible with fluorescent probes. This opens new and exciting possibilities for the measurement of weakly expressed genes or small expression modulations such as encountered in the brain, which were up to now not accessible with the current fluorescent techniques.



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