

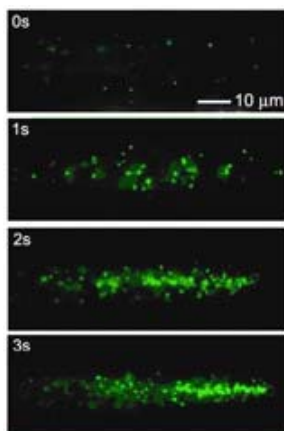
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Molecules queue up to be detected

Very small amounts of molecules such as DNA can be detected using single-molecule fluorescence. Thanks to microfluidic manipulations, the method can now be made quantitative and efficient.

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Time-serial epifluorescence images of flowing DNA, gradually focusing on the centre of the downstream microchannel after a potential was applied. Adapted figure reprinted with permission from ref. 1. Copyright (2005) American Chemical Society.

the polymerase chain reaction (PCR) to amplify the DNA or RNA in the sample so that it can be assayed more readily. But as well as introducing extra steps into the measurement process, PCR isn't always reliable. For example, the background 'noise' of other nucleic acid molecules gets amplified too.

Wang and colleagues have sought a detection method based on identifying fluorescent signals from single molecules. A great many previous studies have established that individual molecules can be detected this way. Non-fluorescent molecules can simply be labelled with fluorescent markers or 'molecular beacons'.

A device for channelling molecules into a narrow passage, where they can be detected one by one by fluorescent labelling, has been developed by US researchers¹.

Tza-Huei Wang, of John Hopkins University in Baltimore, and co-workers say that their technique can be used for fast, accurate and quantitative measurement of DNA and RNA molecules with specific sequences, without the complications and uncertainties of current sequence-specific detection methods.

Techniques capable of providing this kind of information are becoming central to medical diagnostics, studies of systems biology, and drug discovery. For example, it should be possible to provide early diagnosis of some cancers if the RNA molecules transcribed from genes associated with the disease states can be detected from individual cells.

Typically, all of these applications require measurements on only tiny quantities of material. One way of coping with this is to use

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That, however, isn't a complete solution for making quantitative studies of molecules in small quantities. Imagine trying to measure the concentration of a fluorescently labelled molecule in a sample, for example using confocal fluorescence microscopy to detect each glowing molecule.

The confocal technique involves shining a focused laser beam through the sample and detecting the fluorescence photons emitted as a result. But molecules are detected only when they happen to drift into the focal plane of the laser spot. Most of the molecules remain undetected at any moment, and the only way to get a good measure of concentration is to perform the measurement for a long time to determine a reliable average.

Wang and colleagues have set out instead to focus all the fluorescent molecules into the small detection region illuminated by the laser beam – rather like counting a crowd of unruly schoolchildren by getting them to pass one by one through a doorway. To achieve this focusing, they have used microfluidic technology.

The researchers cut a channel 5–10 μm deep and 40 μm wide in a silicon wafer. This channel guided the tiny volume of sample past the laser beam used for detection. But the molecules or particles in the sample solution were focused even more tightly using metal electrodes deposited on the floor and walls of the microchannel.

Wang and colleagues applied a.c. and d.c. electric fields to these electrodes such that dielectrophoretic and electrophoretic forces brought the particles to sit above the central electrode. Electrophoresis is the movement of charged particles (such as nucleic acids) in response to a static electric field; dielectrophoresis acts also on uncharged particles, owing to electrical polarization induced by the fluctuating (a.c.) field.

These forces not only help to bring the particles into a narrow, virtually single-file line, but they also confine the particles to the laser's focal plane, making sure that each of them is brightly illuminated and clearly visible.

The researchers showed that both latex microspheres and fluorescently labelled DNA molecules were individually visible in the device when focused in this way. As they passed through the detection region, each particle emitted a burst of photons that could be clearly distinguished above the background level by sensitive photodetectors. These bursts became stronger and more frequent when the focusing fields were applied, relative to when they were switched off, reducing the time required for an accurate quantitative measurement by around a factor of 50.

Another key to making the technique quantitatively accurate for fluorescently labelled molecules is to ensure that unattached labels in the sample do not get detected. For labelling specific sequences of DNA or RNA, the molecular beacons are single strands of nucleic acid complementary to the target sequence, with fluorescent dyes attached. These labels will pair up with the target sequence and no other.

Wang and colleagues used a clever trick to turn unpaired labels 'off'. To one end of the label strand they stuck the fluorescent group (chromophore); to the other end they attached a quencher group that suppressed fluorescence if it was close enough to the chromophore. And as well as the target-recognition sequence, the researchers gave their label strands short sequences at each end that paired with each other, bending the unattached label molecules into a hairpin loop in which the chromophore and quencher are adjacent. Only when these labels pair up with a target strand are the two groups prised apart so that the fluorescence is switched on.

The researchers find that their microfluidic sensor can measure concentrations of 0.7 nanomolar with 99 per cent accuracy in under a second. The same accuracy can be achieved for concentrations a thousand times smaller by extending the measurement time to 90 seconds. They say that the technique also has the advantage of requiring only minuscule

amounts of the molecular beacons – typically about 1.4 attomoles (10^{-15} moles). This is important, because the cost of these molecular probes commonly accounts for a considerable part of the total cost of assays of this kind.

References

1. Wang T.-H., Peng Y., Zhang C., Wong P. K. & Ho C.-M. Single-molecule tracing on a fluidic microchip for quantitative detection of low-abundance nucleic acids. *J. Am. Chem. Soc.* **127**, 5354–5359 (2005)

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