MUSE, Lab on Chip for chemical analysis in-situ.

F. Eckhard[1], S. Arnoult[1], C. van Driel[1], G. Hoogakker[1], P. Kiewiet[1], D. van Loon[1], R. Heideman[2], R. Mateman[2], A. Prak[2], M. Wehrmeijer[2], D. van den Assem[3], R. van Benthem[3].

[1] Stork Product Engineering, Amsterdam, the Netherlands;
[2] LioniX, Enschede, the Netherlands;
[3] National Aerospace Laboratory NLR, Emmeloord, the Netherlands

ABSTRACT

For a number of years in the Netherlands work has been done on the development of a system for capillary electrophoresis (CE) applicable in a space environment (Eckhard et al., 1997). A few years ago, the development of MUSE (Multi-purpose micro analysis tool) started. For this development the so-called MATAS technology, which enables the design and development of compact and rugged "Lab on Chip" systems, is applied. For the first system, fluorescence detection has been selected. In a later stage the development of an electrochemical and a refractive index detection system, started. Raman Spectroscopy is studied as an additional detection technique too. Opposite to fluorescence detection, these other detection techniques do not need sample pre-treatment, resulting in a very compact layout of the overall system, and enabling the system to become part of a planetary rover. An example of such system is worked out and presented.

In the search for the origin of life, chemical analysis plays an important role. Many important molecules required for life exist in two forms. These two forms are non-super imposable mirror images of each other, i.e.: they are related like our left and right hands. Hence this property is called chirality, from the Greek word for hand. Nearly all-biological polymers must be homochiral to function. Capillary electrophoresis has emerged as a high-resolution analytical technique for the separation of chiral molecules, as it is simple to construct and modify a chiral environment within a capillary.

INTRODUCTION

The principal goal of research in the area of the cosmic evolution of bio-organic compounds is to determine the history of the elements carbon, hydrogen, nitrogen, oxygen, phosphorus and sulphur from their birth in the stars to their incorporation into planetary bodies. Therefore it is of importance to determine what chemical systems could have served as precursors of metabolic replicating systems both, on Earth and in situ, the planetary surface.

Many important molecules required for life exist in two forms. These two forms are non-super imposable mirror images of each other, i.e.: they are related like our left and right hands. Hence this property is called chirality, from the Greek word for hand. The two forms are called enantiomers (from the Greek word for opposite) or optical isomers, because they rotate plane-polarised light either to the right or to the left.
Nearly all-biological polymers must be homochiral (all its component monomers having the same handedness. Another term used is optically pure or 100 % optically active) to function. All amino acids in proteins are 'left-handed', while all sugars in DNA and RNA, and in the metabolic pathways, are 'right-handed'.
A 50/50 mixture of left- and right-handed forms is called a racemate or racemic mixture. Racemic polypeptides could not form the specific shapes required for enzymes, because they would have the side chains sticking out randomly. Also, a wrong-handed amino acid disrupts the stabilising α-helix in proteins. DNA could not be stabilised in a helix if even a single wrong-handed monomer were present, so it could not form long chains. This means it could not store much information, so it could not support life.

The origin of the homochirality of biological molecules has puzzled scientists since the chirality of molecules was discovered by Louis Pasteur more than 150 years ago. The discovery of an excess of L-amino acids present in the Murchison and Murray meteorites indicating that there is a preference for L-amino acids in solar system material. This supports an idea, first proposed by Rubenstein et al., for an extraterrestrial origin for homochirality (Rubenstein et al., 1983).

Upcoming strategies for Mars exploration will require in situ analyses by instruments that are orders of magnitude more sensitive than the Viking GC/MS. One such instrument, the Mars Organic Detector (MOD), has originally been selected for the 2005 Mars Lander mission (Kminek *et al.*, 2000). MOD is able to detect amino acids and polycyclic aromatic hydrocarbons (PAH's) by sublimation and fluorescence detection. MOD is a "yes-no" detector for the presence of amines and PAH's. There are plans for the incorporation of a capillary electrophoresis (CE) system. CE together with GC/MS and liquid chromatography are the techniques to enable the analysis of chiral molecules.

CAPILLARY ELECTROPHORESIS

The capillary electrophoresis analysis technique is based on the principles governing the relative mobility of analytes solution within capillaries (ID <= 200 µm) under the influence of an electric field (E <= 30 kV). Selectivity can be controlled by altering electrolyte properties such as pH, ionic strength, electrolyte type and electrolyte additives, e.g. surfactants, divalent amines, charge reversal reagents and zeta potential suppressers. Detector options for capillary electrophoresis currently include ultraviolet (UV) and diode array spectrophotometric, fluorescence, laser induced fluorescence, electrochemical and mass spectrometric detectors.

CE consists of a family of techniques, which operate on intrinsically different separation mechanisms, each offering specific advantages. For example, using micellar electrokinetic capillary chromatography (MEKC) in which a micelle-forming surfactant solution is added to the electrolyte also non-ionic species can be analysed. These techniques differ in the way of processing and can be performed using the same hardware configuration, which makes CE to a very broadly applicable analysis technique. In Table 1 an overview of the CE application areas is presented.

Organic compounds	Interaction studies
Inorganic cat- and anions	Enzyme reactions
Nucleotides and oligonucleotides	Diagnostics
Peptides	Drugs, pharmaceuticals and their metabolites
Proteins	Chiral separations
Amino acids	Preparative applications
Cells, viruses and bacteria	Food analyses
Polymer and particle analyses	Environmental analyses

Table 1: Overview of application areas for capillary electrophoresis.

MUSE

In the second half of 2000 the development of the MUSE: Multi-purpose micro-analysis tool started. For this development the MATAS: Modular Assembly Technology for µ-TAS has been applied (Wissink et al., 2000). MATAS is a simple packaging and assembly technology, which enables the development of compact MEMS tools. In Figure 1 the principles of this technology are showed. In Figure 2, an example of a so-called electric tongue, a sensor array which enables to recognize different fluids.

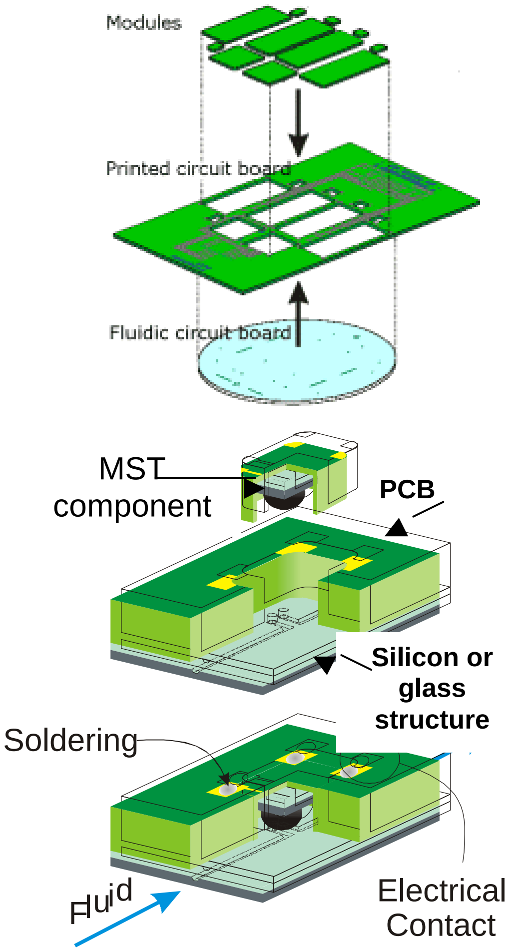

Figure 1: In the MATAS concept the sensors or other MST parts are packaged using standard PCB technology. The PCB taking care for all electrical connections is bonded with a silicon or glass structure enabling all fluid connections. The packaged MST parts form replaceable and testable components. The technology is applicable to a large variety of MST parts from various suppliers.

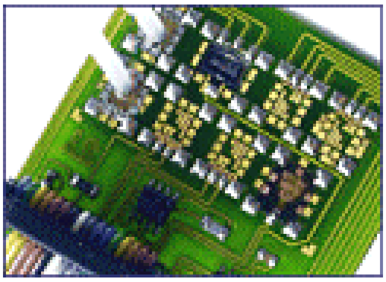

Figure 2: Picture of a so-called electrical tongue developed using MATAS. This sensor array enables to recognize different fluids.

The basis of MUSE is formed by a planar glass system containing the capillary as described first by Manz and Widmer (Effenhauser, 1993). This planar glass system is constructed of two plates. In the lower plate the channels are etched. A second glass plate is covering this channel system. In the cover plate holes are drilled to reach the channel system. This planar glass system in combination with the MATAS technology the MUSE design enables fluid management in a low gravity environment. In Figure 3 the layout of the planar glass system is showed.

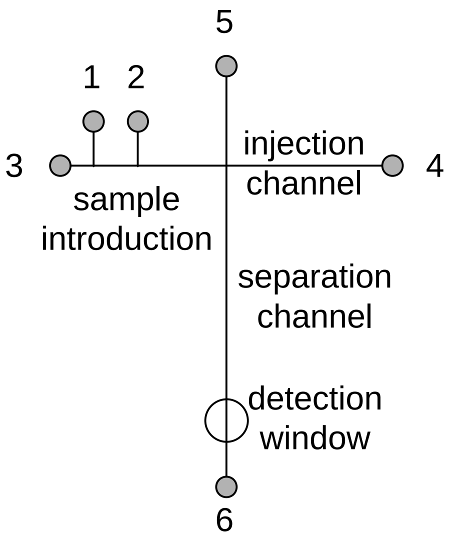

Figure 3: Layout of the separation system 1-2 -Sample introduction; 3-4 - Injection; 5-6 - Separation.

To prevent gas formation at the electrodes in case voltage is applied, dedicated electrodes have been developed which produce no gas during operation. In Figure 4 MUSE is presented.

At this moment three detectors are under development for MUSE:

- Fluorescence
- Refractive index
- Electrochemical.

Currently, the most widely used detection method in CE is UV absorption, but an UV detector is concentration sensitive which causes problems upon decreasing detection cell dimensions. In MUSE the path length 12 µm. Furthermore, Pyrex glass has been used for the development of the channel system and glass has a strong absorption in the UV wavelength area.
Fluorescence detection is more sensitive and selective than UV detection. A diode laser is used in the MUSE set-up.

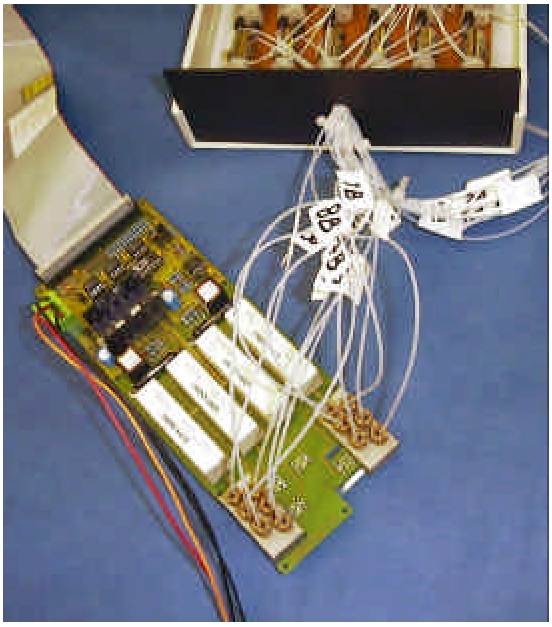

Figure 4: The "MUSE system". At the development of the system, COTS (commercially available) electronics has been applied. The flight hardware will be up to 50% smaller.

Upon the construction of the detector on the MUSE system, some problems have been encountered. Due to the very compact layout of MUSE, interfacing is difficult. Therefore the option of using light pipes has been investigated. In Figure 5 these light pipes are showed.

Figure 5: Detail of the channel system. The V shaped channels, forming a light pipe, is filled with a fluid and connected using optic fibers with the fluorescent detector.

Disadvantage of fluorescence detection is the fact that the sample has to be bound to a fluorophore. Although, in the former CAELIS project, a completely automated sample pre-treatment sub device has been developed which can easily be combined with the MUSE chip, it complicates the system considerably (Eckhard et al., 1997). This could be a problem when the system would be used in a planetary rover. Therefore, two parallel projects on the development of two other detectors have been started in the first half of 2002. An electrochemical detector and a detection system based on refractive index are being developed.
The design of these new detectors is in accordance with the MATAS philosophy, they are modular assembled.

It is possible to develop a glass channel system incorporating both detectors using photolithographic techniques, however, this would make the system rather expensive, and stunt the earthbound usage. For both new detectors the fluid has to get in contact with the detection system. To prevent the introduction of dead volumes and/or distortion of fluid flow in the separation channel, as it has been proven that bends or changes in the dimension influence the separation negatively, a new glass channel structure has to be designed. In the first set-up there an etched channel system covered with a thin glass sheet was chosen. In the latest design a thin sheet of silicon nitride covers the channel system. The silicon nitride sheet is a few µm thick resulting in limited curves in and broadening of separation channel and a low dead volume.

The refractive index detector is based on Mach-Zehnder interferometer developed using planar wave-guide technology (Heideman, R. 1993).

Figure 6: Photo of the Mach-Zehnder interferometer (Photo: Mierij Meteo).

This hybrid opto-electronic sensor device is constructed of a wave-guide structure consisting of a high refractive index SiON wave-guide layer, surrounded by lower index layers. Locally a sensing window is applied.
In Figure 7 a principle sketch of a planar wave-guide is presented. The light wave channel of e.g. a slab wave guide consists of a thin core layer with a thickness in the range of 0.1 to 5 µm applied on or in a substrate by micro technologies such as deposition, sputtering, chemical vapour evaporation, diffusion, etc. Confining the light in the lateral direction makes a light wave channel.
For optical sensing light is needed. This light can be supplied by a chemical reaction, chemo luminescence (generator type sensor), or by an external light source (modulator type). In modulator type chemo-optical sensors the species X in the vicinity of the sensing area induces a change of an optical parameter, which modulates a guided wave that propagates through the sensing part (Lambeck, 1992).

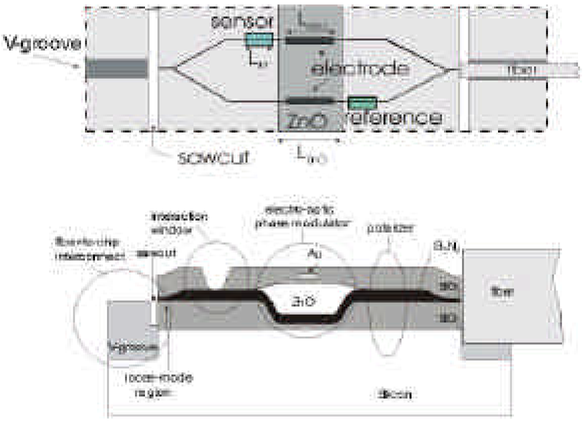

Figure 7: The principles of the Mach-Zehnder interferometer. The Integrated Mach-Zehnder Interferometer is connected to chip mounted fibers. The measurement essentially determines the index of refraction of a gas specific absorbent material. An incoming coherent monochromatic light beam is split into two beams of equal intensity. For a perfect interferometer the transfer function I_{in}/I_{out} is determined only by the phase difference φ between both beams when meeting again: $I_{in}/I_{out} = \cos^2 \varphi/2$. Changes in the local dielectric constant in one of the branches will be manifested as a change in the phase φ.
By locally applying a chemo-optical transduction layer on top of one of the branches, we obtain a chemo-optical sensor which action can be presented by the transduction chain:
$\Delta C_x \rightarrow \Delta\varepsilon' \rightarrow \Delta\beta' \rightarrow \Delta\varphi \rightarrow \Delta(I_{in}/I_{out})$.

For this purpose the channel region has to have an effectively higher refractive index than all its surroundings. Generally the width of the channels is in the range of 2-10 µm and like optical fibres the light can follow the channel even if it bends.
A field in a guide is not strictly confined to the core layer, but also shows exponentially decaying tails in the surrounding material, i.e. the evanescent fields. The decay length ranges from one tenth of the applied wavelength to infinity.
Such wave-guide can be applied as sensor using the evanescent field for probing the changes in optical properties at the sensing area. In Figure 8 the principles are shown.
This sensing by modulation can be based on linear or non-linear phenomena. Non-linear phenomena are for example Raman effect, and luminescence. With these effects the angular frequency ω_0 of the guided mode after the sensing area differs from the frequency ω_I of the incoming mode. In linear sensors the frequency remains constant, but the polarisation state, amplitude or phase of the guided mode is changed as a consequence of the concentration-induced change of the dielectric constant of one of the compositional materials.

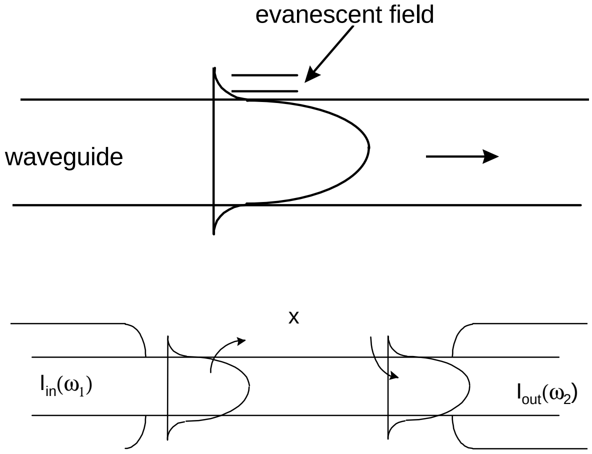

Figure 8: Measurement principles of a dielectric sensor
top: Field profiles of slab guide modes 0 and 1
bottom: Sensing part of a non-linear sensor.

The conductivity sensor is a micro engineered sensor that measures the electrolytic conductance of a liquid. State-of-the-art conductivity sensors are usually based on impedance measurement by means of metallic electrodes configured in a relatively large measurement cell. The planar micro sensor consists of thin film metallic interdigitated (“finger”) electrodes coated with a thin insulating layer of extremely inert tantalum oxide. This oxide layer has been specifically developed for the ion-sensitive field effect transistor ISFET, a micro sensor, which measures the pH of a liquid.

In conventional conductivity sensors the interface between liquid and metal suffers from interferences caused by redox processes, whereas the liquid - oxide interface of the micro sensor is very stable and well defined. This allows accurate measurement in complex and demanding solutions, while in addition the inert coating will stand aggressive liquids with low or high pH.

In Figure 9 a picture of the conductivity sensor is presented.

CHARACTERISTICS AND USE

In Table 2 some characteristics of the MUSE breadboard are presented. As at the development of the present breadboard, for the non-critical hardware parts commercial available hardware has been applied, dimensions, mass, etc. are estimated.

For the present breadboard, a dedicated fluid management system has been developed which enables to test all different analysis scenarios (see also Figure 4).

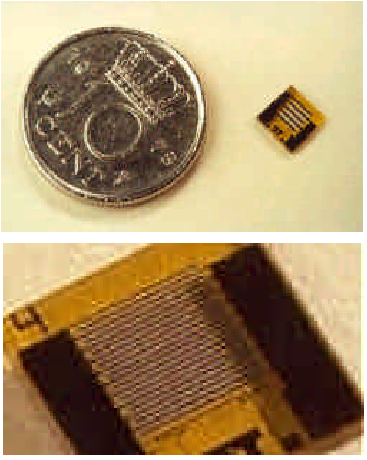

Figure 9: The conductivity sensor, which is applied in MUSE (Photos: Lionix). (The old Dutch ‘dubbeltje (top) has the dimensions of about a piece of 10 Eurocents).

Figure	**Dimension**
Injection volume[1]	7.2 ml^{-6}
Sample volume	1.2 ml^{-3}
Injection voltage	2000 V
Separation voltage	<= 5000 V
Effective field strength	675 $V.cm^{-1}$
Analysis time	<= 100 sec.
Volume[3,4]	<= 2 dm^3
Mass[3]	1.2 kg
Power[3]	<5 W

Table 2: MUSE characteristics. The presented characteristics are an indication only because the system is still under development.
[1]) The volume which is analysed; [2]) Volume introduced on the system; [3]) Estimate; [4]) The overall dimensions are defined by the detector(s) applied. The fluorescence detection system will have the largest dimension.

To serve future planetary missions the European Space Agency (ESA) has started the development of concepts for micro-rovers. Within the Technology Research Program (TRP) activity "Micro-Robots for Scientific Applications", an interdisciplinary group of space companies and research labs have investigated new designs of micro-rovers. Two concepts, a simple and robust one called Nanokhod (see Figure 10) and a more innovative one called SpaceCat, have been selected by ESA (Siegwart et al., 1998).

For the Nanokhod a first concept of MUSE as a payload has been worked out. This rover, which is manufactured by Von Hoerner & Sulger and the Max-Planck-Institute for Chemistry, is a simple and robust, tracked rover with 4 degrees of freedom, and a stowage volume of 2 dm^3. In Figure 11 this concept is shown.

Figure 10: The Nanokhod developed by von Hoerner & Sulger and Max-Planck-Institute for Chemistry, in cooperation with ESA (Betrand et al., 1998)(Image courtesy vH&S).

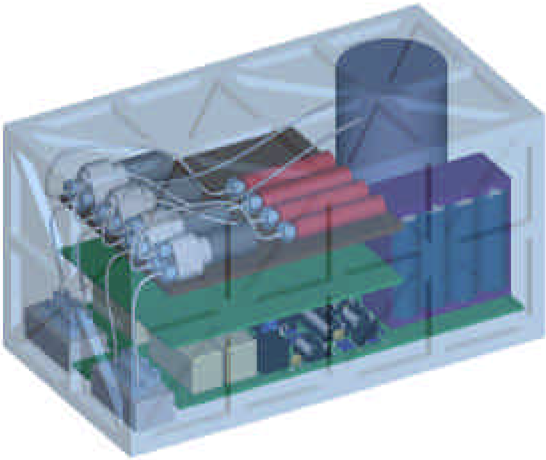

Figure 11: Concept of MUSE as Nanokhod payload for exploration of e.g. the Mars surface.

Being an analytical tool with broad applicability, low in consumables, MUSE can also be serving as tool to support onboard research on the International Space Station.

STATUS AND FUTURE PLANS

Being a development project, the MUSE design and development has encountered a number of problems. Apart from the financial ones, most have been tackled and it is expected that mid 2003 the test with MUSE and the integrated conductivity detector can be started while it is planned to have the first results of the fluorescence detector at the end of 2003.
Problems with the incorporation of the refractive index sensor have slowed down the development of this detection system considerably, but the team is convinced that this problem can be solved too.

With MUSE, a powerful tool has been developed, not only for use in space but also for ground based analysis in clinical and other environments.
The use of CE is still not wide spread yet. CE is about two decades commercial available. However, the lack of knowledge and acceptance of CE methodology are for the time being aspects that limit general usage. The presently available commercial hardware has to be made suitable for high throughput screening, a necessity for today's laboratories. Besides this CE often falls short towards sensitivity compared to GC and HPLC, both latter techniques have optimized detection limits around 10 times better than CE. Nevertheless, however, by the application of Microsystems Technology and MATAS, to design the new generation CE hardware most of these drawbacks might be easily nullified. Especially for use in space, this miniaturized CE hardware has tremendous advantages.
The availability of more detector systems enables the simultaneous analysis of organic and inorganic molecules, thus, for example, the search on pre-biotic life and the soil in which the organics are found.
Furthermore, as a result of the increasing development on miniaturized optical systems (MOEMS) the development of a detection system based on Raman Spectroscopy is studied to widen the possibilities of MUSE.

ACKNOWLEDGMENT

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ABBREVIATIONS

CAELIS	Capillary Electrophoresis In Space
CE	Capillary Electrophoresis
COTS	Commercial Of The Shelf
ESA	European Space Agency
GC	Gas Chromatography
HPCE	High Performance Capillary Electrophoresis
HPLC	High Pressure Liquid Chromatography
ID	Inner diameter
IDP	Interplanetary Dust Particle
ISS	International Space Station
LIF	Laser Induced Fluorescence
MATAS	Modular Assembly Technology for µ-TAS
MEKC	Micellar Electrokinetic Chromatography
MEMS	Micro Electro Mechanical System

MOD	Mars Organic Detector
MS	Mass Spectrometry
MST	Micro System Technology
MUSE	Multi-Purpose µ-Total Analysis System
NASA	National Aeronautics and Space Administration (USA)
NIR	Near Infra Red
NIVR	Netherlands Agency for Aerospace Programs
NLR	National Aerospace Laboratory
PAH	Poly Aromatic Hydrocarbons
PCB	Printed Circuit Board
SION	Silicon Oxide Nitride
TRP	Technical Research Programme
UV	Ultra Violet
VIS	Visible
µ-TAS	Micro Total Analysis System

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CONTACT

The contact for these activities is:

Mr. F. Eckhard
Stork Product Engineering B.V.
P.O.Box 379
1000 AJ Amsterdam
The Netherlands
Phone: +31 20 5563 488
Fax: +31 20 5563 556
e-mail: fir.eckhard@stork.com
Internet: www.stork.com/spe