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Scaling and the design of miniaturized chemical-analysis systems

Dirk Janasek[1], Joachim Franzke[1] & Andreas Manz[1]

Micrometre-scale analytical devices are more attractive than their macroscale counterparts for various reasons. For example, they use smaller volumes of reagents and are therefore cheaper, quicker and less hazardous to use, and more environmentally appealing. Scaling laws compare the relative performance of a system as the dimensions of the system change, and can predict the operational success of miniaturized chemical separation, reaction and detection devices before they are fabricated. Some devices designed using basic principles of scaling are now commercially available, and opportunities for miniaturizing new and challenging analytical systems continue to arise.

In chemical engineering, problems frequently arise in scaling up chemical processes. Research is normally conducted in glassware on the millilitre scale, whereas cubic-metre capacities are required for production. The main scale-up problems are associated with heat and mass transport, and can result in increased formation of by-products and lower yields. In the worst cases, shortcomings can lead to runaway, in which the rate of heat generation exceeds the rate of cooling available, and other hazardous situations.

Some of the scaling laws described here were developed between the 1880s and the 1930s in the growing field of engineering. The aim was to provide a framework for engineers to establish how material would behave on different length scales, allowing them to optimize output and minimize the risk of runaway and other hazards. In the pre-computer era, these laws proved to be simple and useful tools, particularly when the engineering mathematics required to model a chemical process became complicated.

During the past 20 years, microfluidics, micrometre-scale total analysis systems (μTAS) or so-called 'lab-on-a-chip' devices have revived interest in these scaling laws and dimensionless groups for downscaling purposes[1]. Such devices have a range of practical benefits (see page 368).

Here, we aim to review some of the important principles that contribute to the design of novel μTAS and to propose future research activity, to inform the reader, who might ultimately use such devices for research, and even to inspire the reader to take on the challenge of designing new μTAS.

For simplicity, we use three miniaturized devices as the main examples for discussion: an open-tubular chromatographic system that is used to separate molecules from mixtures (Fig. 1); a microwell plate as an example of a device in which chemical interactions must be optimized; and a gas-phase detection device that uses a glow-discharge plasma (Fig. 2).

The scaling laws considered here are generally not valid on the nanometre scale, so 200 years' experience in chemistry cannot be applied in this case. We are not yet convinced that practical applications of nanofluidics are feasible; however, there seems to be tremendous potential for basic research on the subject. We consider the absolute limits of the principles described here for the scaling down of chemical processes.

Separation

The measurment of a specific compound in a complex sample matrix can be carried out using selective (bio)chemical sensors or non-specific detectors applied after the separation of the sample mixture into distinct zones of the single analytes. The most widely used separation principles are chromatography (based on the different distributions of the compounds within a complex mixture between two phases) and electrophoresis (based on on the differential movement of charged components in an electric field).

Figure 1 | Examples of the miniaturization of separation techniques. The figure compares the size of a commercial gas chromatograph and column with that of a microscaled column on a chip. (Photo of microscaled column courtesy of J. Müller, Institut für Mikrosystemtechnik, Technische Universität Hamburg-Harburg, Germany.)

Some of the initial theoretical work on scaling down devices was in the field of chromatography and was published as early as the 1950s — for example, by Golay on open-tubular gas chromatography[2] and van Deemter *et al.* on packed-column liquid chromatography[3]. These papers led to the development of commercial gas chromatographs using capillaries with micrometre diameters, and liquid chromatographs with micrometre-scale particles for the stationary phase.

The first commercial microfluidic-based platform for the analysis of DNA, RNA, proteins and cells — known as the 2100 Electrophoresis Bioanalyzer — was launched in 1999 by Agilent. Based on the LabChip technology from Caliper LifeSciences, it provides good-quality data quickly and accurately and is an alternative to labour-intensive gel electrophoresis. Using one platform, the integrity and purity of RNA can be determined in less than 30 min, 16 samples of RT–PCR (polymerase chain reaction with reverse transcription) products can be quantified in the same time range, the transfection efficiency of cloning experiments

[1]ISAS — Institute for Analytical Sciences, Bunsen-Kirchhoff-Str. 11, D-44139 Dortmund, Germany

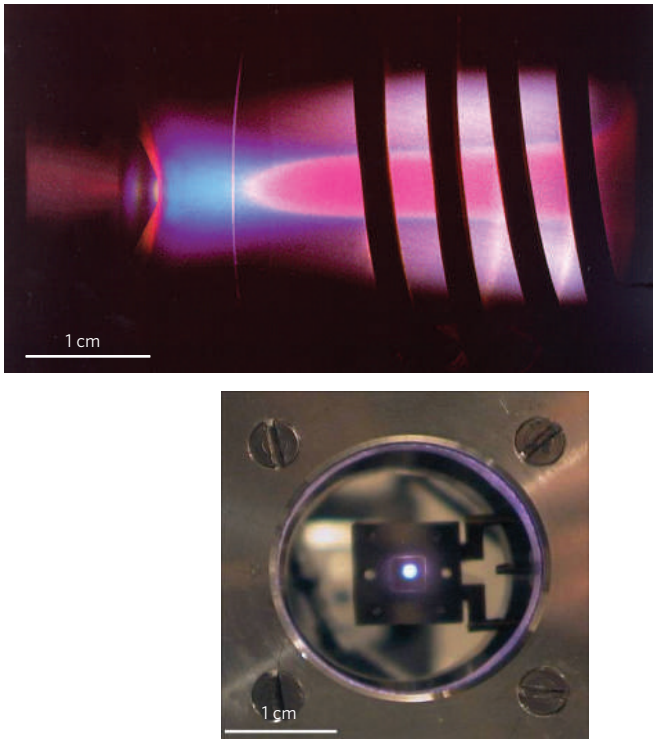


Figure 2 | Plasma detectors, an example of miniaturization in detection. Photographs of an inductively coupled plasma with power consumption of 1 kW and a microhollow cathode plasma of less than 1 W. The whole of the microhollow cathode has a diameter of 100 μm.

can be monitored, and the size and concentration of expressed proteins can be analysed. The success and reliability of the 2100 Bioanalyzer can be estimated from the number of publications in which the system has been used. Since its launch, the number has increased exponentially and reached 150 per month in 2005 (G. P. Rozing, personal communication). The device demonstrates the success of the application of scaling laws in chemical separations.

Two approaches have proved particularly useful in the design of miniature separation devices, and these are discussed below.

Dimensionless-parameters approach

'Dimensionless parameters' define units such as volume, column length, linear flow rate, retention time and pressure drop in terms of quantities that can be assumed to be constant over the entire system. For decades, engineers have used dimensionless parameters to correlate experimental results when large numbers of significant variables are involved.

In the case of chromatography using an open-tubular column (in which the columns are not packed, but instead the walls of the column support the stationary phase and a hollow core allows the mobile phase to move freely), the constant quantities include inner diameter, mobile-phase viscosity, average diffusion coefficient of a sample in the mobile phase, and Poiseuille number for a circular cross-section. The dimensionless-parameters approach can be applied as long as the contribution of other physical phenomena not considered in the parameter can be neglected.

Nonlinear behaviour can be usefully described with the aid of dimensionless parameters[4] such as the Peclet number, ν, which is the ratio of axial bulk flow to diffusion mass transport (effectively describing the relative importance of convective or diffusive transport, or change in flow rate), the Fourier number, τ, which describes the average number of times a molecule contacts the wall of the capillary (elution time, or mixing efficiency), and the Bodenstein number, Π, which characterizes the backmixing within a system (or pressure drop). These parameters make it possible to extrapolate results obtained for one system to other similar systems through multiplication by constant factors, as shown in Table 1. So, even before starting an experiment, we can establish whether or not the device will function. For instance, miniaturization to channel sizes of 1.5 μm results in a pressure drop of 3,900 bar, which is difficult if not impossible to provide. An example of how dimensionless variables can be applied to capillary separation systems is provided by Knox and Gilbert[5], who derived sets of optimal conditions for capillary liquid chromatography using reduced parameters.

The Reynolds number, Re, is a particularly useful dimensionless parameter. It represents the ratio of inertial forces ($\nu \cdot \rho$, where ν is the average velocity and ρ the fluid density) to viscous forces (η/d, where η is the dynamic viscosity and d the characteristic length, such as the diameter of the capillary); it characterizes the fluid flow as being laminar or turbulent[6]. Once an experiment indicates that a transition will occur, say at Re = 2,000, the outcome of any other experiment and the behaviour of the flow can be predicted. Because the Reynolds number is proportional to the characteristic length d, for miniaturized systems, a small value will be obtained, indicating laminar flow. Such parameters are useful for controlling separations and for mixing to optimize the output of chemical reactions (see below).

Some behaviour on the nanometre scale can be estimated by applying these particular scaling laws, and the results used as a means of testing whether answers derived by more sophisticated methods are reasonable. Perhaps surprisingly, many scaling laws for the most mechanical systems are quite accurate on the nanometre scale, but in electromagnetic systems many scaling laws fail dramatically. Scaling laws for thermal systems have variable accuracy[7].

Scaling relationships based on classical continuum models ultimately break down as a consequence of atomic-scale structure, mean-free-path effects and quantum-mechanical effects.

Similarity approach

Useful information about the behaviour of simple miniaturized flow systems can be attained by considering the proportionalities within a system. The parameters of interest, such as flow rate, pressure drop or electric field, are viewed as a function of the variables to be miniaturized in space and time. Using this approach, the major trends

Table 1 | Examples of the dimensionless-parameters approach

Parameters	Diameter, d_c (μm) 30	5	1.5	Dimensionless parameters Relationship	Calculated values
Length, L (m)	13.5	2.3	0.67	Reduced column length, $\lambda = L/d_c$	450,000
Time, t (min)	122	3.13	0.28	Fourier number (reduced retention time), $\tau = t \cdot D/d_c^2$	7,500
Pressure difference, Δp (bar)	10	350	3,900	Bodenstein number (reduced pressure drop), $\Pi = p \cdot d_c^2/\Phi \cdot \eta_m \cdot D_m$	280
Peak standard deviation in terms of length, σ_X (μm)	13,400	2,225	670	Reduced variance of elution profile in terms of length, $s_X = \sigma_X/d_c$	450
Peak standard deviation in terms of time, σ_t (ms)	6,750	188	17	Reduced variance in terms of time, $s_t = \sigma_t \cdot D_m/d_c^2$	7.5
Peak standard deviation in terms of volume, σ_V (pl)	10,200	47	1.3	Reduced variance in terms of volume, $s_V = \sigma_V/d_c^3$	350

Calculated parameter sets for an open-tubular column liquid chromatography (LC) system with one million theoretical plates at zero retention as capillary diameter changes (Peclet number, ν = 38). The diffusion coefficient is assumed to be $D_m = 10^{-9}$ m^2 s^{-1}; the viscosity is $\eta_m = 10^{-3}$ Ns m^{-2}; and the Poiseuille number, Φ, has a value of 32 for a circular cross-section. An open-tubular LC system with zero retention was chosen for numerical simplicity. $_m$, molecular.

a parameter undergoes during its downscaling become apparent, with no knowledge of material constants (such as viscosity or heat capacity) being necessary.

For any system, it is possible to proceed from a given point, such as an experimental result, and extrapolate to estimate the magnitude of the variables in a downscaled system. Changes in geometry only influence this estimation by a constant factor. Although this approach cannot be used to predict the feasibility of a system, it can, in principle, allow physically impossible cases to be rejected and provide an idea of the order of magnitude of relevant variables. If it is assumed that miniaturization is a simple three-dimensional downscaling process characterized by a typical length parameter, d (refs 1, 8), we can easily predict the behaviour of the relevant physical variables. The typical length d represents the scaling factor of the miniaturization. A single degree of freedom for the mechanical parameters remains: time. For simplicity, only two important cases are considered and discussed below.

If the timescale is the same for the miniaturized system as for the full-scale system, it is referred to as a time-constant system (Table 2). Relevant time variables (such as analysis time, transport time and response time) remain the same. However, linear flow rate in a tube would decrease by a factor d, volumetric flow rate by d^3, and the Reynolds number by d^2. By contrast, the pressure drop needed to maintain the desired flow rate would remain the same. This time-constant behaviour is important for simple transportation and for analytical techniques such as flow-injection analysis (FIA) systems. Diffusion would certainly have a more dominant role in mass flow in miniaturized systems. The main advantage in downscaling simple transport or FIA systems lies in the conservation of carrier and reagent solutions. A 10-fold decrease in size, for example, would cause a 1,000-fold decrease in carrier or reagent consumption.

The diffusion-related system (Table 2) becomes important when molecular diffusion, heat diffusion or flow characteristics dictate the separation efficiency in a given system. In such instances, the timescale is treated as a surface that is proportional to d^2. This behaviour is in perfect agreement with standard chromatographic and electrophoretic band-broadening theory. All dimensionless parameters, including the Peclet number, Fourier number and Bodenstein number, remain constant regardless of the size of the system[1]. In other words, hydrodynamic diffusion, heat diffusion and molecular diffusion effects behave in the miniaturized system exactly as in the original system.

This means that downscaling to one-tenth of the original tube diameter reduces related time variables such as analysis time and required response time for a detector to one-hundredth of their original magnitudes. The pressure drop requirements increase by a factor of 100, but the voltage requirements (for electrophoresis or electro-osmosis) remain unchanged. The main advantage of the diffusion-controlled system is that miniaturization achieves faster separations while maintaining comparable separation efficiency. An initial experiment in this field was conducted in 1992 (ref. 9). Finally, the predictions of similarity laws (for example, the same performance of a separation in a shorter time) were experimentally validated when electrophoresis-based separations of amino acids with up to 75,000 theoretical plates could be obtained in about 15 seconds using a microchip system[10]. More recent work on the micrometre scale has confirmed those results for field-inversed electrophoresis[11], chiral separations[12] and free-flow electrophoresis (FFE)[13].

Table 2 | Examples of the similarity approach

Parameter-related system	Time-constant system	Diffusion-related system
Space, x	d	d
Time, t	Constant	d^2
Linear flow rate, u	d	$1/d$
Volume flow rate, F	d^3	d
Pressure drop (laminar flow), Δp	Constant	$1/d^2$
Voltage (electro-osmotic flow),U	d^2	Constant
Electric field, U/L	d	$1/d$
Reynolds number, Re	d^2	Constant
Peclet number (reduced flow rate), ν	d^2	Constant
Fourier number (reduced elution time), τ	$1/d^2$	Constant
Bodenstein number (reduced pressure), Π	d^2	Constant
Reduced voltage, V	Constant	Constant

Proportionality factors are given for mechanical parameters in relation to a characteristic length, d.

FFE is a convenient separation technique with great potential for integration with sample preparation, detection and even chemical reactions in this micrometre-scale regime. In contrast to capillary electrophoresis (CE), in which a longitudinal separation is obtained, in FFE the time domain is converted to a spatial domain. This means that the sample solution can be fed continuously into the separation compartment, separated into its components and supplied to further actions. Applying the miniaturization to FFE devices, the aim of fast acquisition of qualitative and quantitative data can be achieved and has been demonstrated for zone electrophoretic[14–18], isoelectric focusing[13,17,19] and isotachophoretic modes[20]. We envisage that, in the future, two compounds could react in the free-flow device to form the product, which will be separated immediately and continuously from the not-yet-converted starting substances. The product can be fractionated and transferred to subsequent processes — for example, mass spectrometry/mass spectrometry detection — while starting reagents that have yet to be converted can be returned to the inlet of the free-flow device.

By downscaling further into the nanometre range, other physical phenomena have to be taken into consideration, which means that the similarity laws become invalid. As an example, an electric double layer can be considered that is formed as the negative surface charges of the capillary wall are compensated by positive ions from the buffer solution. It consists of a rigid 'Stern' layer in proximity to the capillary surface, and a diffuse layer extending into the bulk solution in the dimension of a few hundred nanometres. If in a nanochannel the electric double layers overlap, the streaming potential will decrease, co-ions will be excluded from the channel, and counter-ions will be enriched. Another phenomenon is the generation of capillary-induced negative pressure during two-phase flow in nanochannels: Tas *et al.*[21] observed a peculiarly shaped meniscus of water plugs in 100-nm nanochannels, which they attributed to a downward bending of the channel capping under the influence of the tensile capillary forces. The interested reader is referred to a comprehensive overview of physical phenomena on the nanometre scale[22]. Articles on the promise of nanotechnology for separation devices[23] and methods of manipulating individual molecules have been published recently (see page 387).

The absolute limit of miniaturization with respect to separation techniques depends on the maximum applicable pressure (which is dependent on the stability of the used material); in the case of chromatography, on the maximum voltage that can be applied in the case of electrophoresis, and on the molecular characteristics that affect, for instance, sterical behaviour, viscosity and surface tension. We emphasize that it is impossible to miniaturize further than to the level of a single molecule.

Chemical reactions

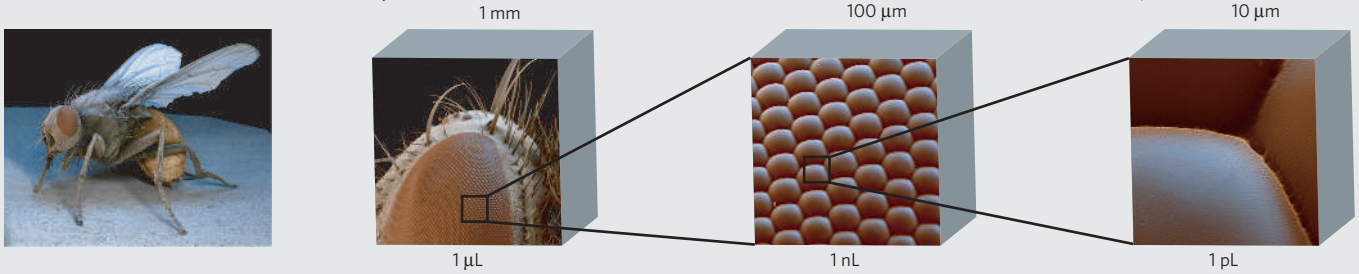
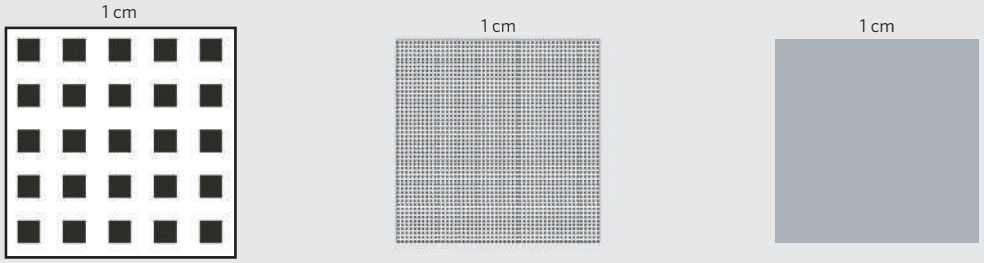
Once the requisite molecule has been separated from its complex starting matrix, manipulation of the molecules for a desired outcome (for example, synthesis or information generation) can be considered.

Miniaturization with respect to reactions first has to take into consideration the fact that a necessary concentration of molecules is required so that they have a chance of colliding and reacting. In addition, forces such as surface tension or adhesion must not prevent the collision of these few molecules.

Mixing

Many reactions, such as bioassays, phase-transfer reactions and multicomponent reactions including PCR and the Ugi reaction (organic

Table 3 | Size dependent diffusion time and information density

Device characteristic	Length, d		
	1 mm	100 μm	10 μm
Volume, V	1 μl	1 nl	1 pl
			
Number of molecules at 1 μM	6×10^{11}	6×10^{8}	6×10^{5}
Diffusion time, $t_d = Dd^2$	15 min	10 s	100 ms
Number of volumes (volumes cm^2)	$5 \times 5 = 25$	$50 \times 50 = 2{,}500$	$500 \times 500 = 25 \times 10^4$
			
Maximum information density	1.5 per min cm^2	250 per s cm^2	2.5×10^6 per s cm^2

A number of device characteristics at three values of the typical length d. Diffusion coefficient $D = 10^{-9}$ m^2 s^{-1}. A housefly is used to provide a pictorial representation of scale. (Reprinted, with permission, from Eye of Science.)

reactions that can be used to form libraries of low-molecular-mass drug-like compounds), are diffusion related. In Table 3, a reaction system of two reactants is described. Usually, two molecules meet by brownian motion, which is dependent on the diffusion coefficient. That meeting process, which is a mixing by diffusion, is greatly enhanced in small structures because the time a molecule needs to travel a distance d decreases as $1/d^2$. Short diffusion times in the millisecond range indicate that an efficient mixing of two solutions will be obtained when they are brought into contact on the micrometre scale. Because there is no turbulence in the micrometre regime, much effort has gone into designing devices to improve mixing capability. A mixing device based on flow lamination[24] and measurements of protein folding by a pH jump[25] used the short diffusion time on the microscale. However, the mixing process can be enhanced further by the use of chaotic advection, as demonstrated by Stroock *et al.* using herringbone mixers[26], and by Song *et al.* for mixing in droplets with a two-phase flow[27].

Multiple reactions

An important consideration in the design of systems to carry out multiple chemical analyses is the number of single, independent devices that can be arranged on a certain area — for example, on a microwell plate. This number increases with $1/d^2$. For instance, with a length of 10 μm, 250,000 devices can be arranged per square centimetre. Such a host of devices could be used in discrete applications, or together for parallel or sequential processing. An impressive example is the multistep synthesis of radiolabelled image probes[28]. Five sequential processes can proceed with high radiochemical yield and purity on the nanogram to microgram scale, and with shorter synthesis time relative to conventional automated synthesis.

Shorter diffusion times increase the exchange of molecular information on the device and therefore the rate of information generation. When this rate is multiplied by the number of volumes present, a maximum information-density number can be defined per time and per surface area. Table 3 shows that it increases with the fourth power of the inverted distance d. It should be noted, however, that this limit cannot be reached in all cases. Slow kinetics, fluid-handling constraints and detection requirements will all restrict information generation. High information densities will be useful in the evaluation of the millions of compounds produced by combinatorial chemistries, or in speeding up clinical DNA diagnostics.

An example is the Affymetrix GeneChip microarray. Semiconductor fabrication techniques, solid-phase and combinatorial chemistry, and molecular biology are integrated to create arrays with millions of probes for DNA–RNA hybridization experiments[29,30]. By 2005, more than 4,200 publications reflected the extensive use of GeneChip systems in many areas, such as sequence analysis, targeted genotyping, expression quantification and regulation, as well as clinical research and molecular diagnostics (information obtained by Affymetrix, 2006).

A second example is the commercially available protein-crystallization chip launched by Fluidigm in 2003. The chip uses micrometre-scale channels based on multilayer soft lithography, and active valves for diffusive mixing of protein and crystallization reagents to overcome two major bottlenecks for protein-structure determination by X-ray crystallography: producing sufficient quantities of material and finding appropriate crystallization conditions. The TOPAZ Crystallizer chip can screen 96 crystallization conditions on four proteins in 384 parallel reactions using just 10 nl of protein per reaction; conventionally, 1 μl per experiment is needed. Here, high-throughput screening and sample-volume requirements reflect the advantages predicted by the similarity laws of scaling.

Detection

Decreasing the dimensions of reaction systems to small volumes with small amounts of analytes naturally stimulates the demand for adequate, high-sensitivity detection techniques. Besides electrochemical methods and mass spectrometry, optical techniques such as absorption, refractive-index variation, chemiluminescence and fluorescence measurements are usually used, because they are non-invasive and provide high temporal and spatial resolution for a suitable experimental set-up[31–33].

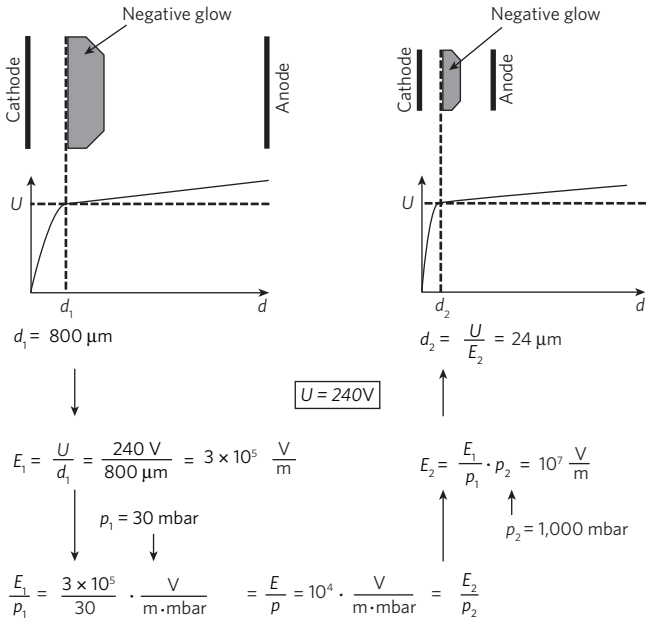


Figure 3 | Similarity relations between glow discharges on the macro- and microscales. Two geometrically similar systems are shown with cathode, anode and negative glow. Underneath, the potential of the discharge is given, depending on the distance from the cathode. The distances d_1 and d_2 are the points at which the discharge voltage change is maximal. Just as d attains values of d_1 or d_2, the breakdown flash occurs, which is where the negative glow of the discharge is located. On the left side is the parameter d_1, obtained with helium direct-current discharge at a pressure, p_1, of 30 mbar. Following the arrows, the calculations show that with the application of a voltage, U, of 240 V and a pressure, p_2, of 1,000 mbar, the distance from the cathode to the negative glow will be diminished to 24 µm. The product $p{\cdot}d$, the ratio E/p (E is the electric field), and all quantities that are functions of $p{\cdot}d$ or E/p, are the same in the two systems. The dependence of the breakdown potential on the product $p{\cdot}d$ was first established by de la Rue and Muller[66] in 1880. Later, Paschen concluded from an extensive study of air, CO_2 and H_2 over a range of values of $p{\cdot}d$ that breakthrough voltage, U_b, is a function $\psi(pd)$ of the product $p{\cdot}d$ only; this result is known as Paschen's law. Carr[67] confirmed its validity for a number of different gases for values of $p{\cdot}d$ from 0.1 Torr·cm to 15 Torr·cm. From typical Paschen[41] curves, E/p is shown to diminish as $p{\cdot}d$ increases. Transformations of quantities in homologous regions are given in ref. 46. In considering similarity for high-frequency fields, the parameter is no longer E/p only, but also involves a time factor that must be changed by a proportionality factor, K. This parameter must contain the term f/p (f is the frequency of the field) as well as E/p.

Optical methods

Fluorescence analysis is particularly attractive because fluorophores can be excited and detected selectively. Furthermore, the excellent sensitivity of fluorescence spectroscopy is greatly enhanced by reducing the size of the detection volume, because the background signal that is generated by impurities of the sample — for example, Rayleigh stray-light and Raman scattering — scales linearly with the size of the detection volume. The fluorescence signal of a single molecule, on the other hand, is independent of the dimensions of the detection volume and remains constant[34,35]. The resulting high signal-to-noise ratio facilitates the recognition of single fluorophores residing in the detection volume. However, with fewer than 10 molecules, the measurement would not allow a statistically covered analysis but give a digitalized output.

Assuming a molecular weight of about 50,000, analyte concentrations in the higher parts per million range are needed for detection volumes lower than a cubic micrometre. At this concentration, effects such as viscosity and light scattering have to been taken into consideration. Detection methods such as surface plasmon resonance or detections related to evanescence fields do not depend on geometrical scaling but on the wavelength of the light; however, they are subject to the same restrictions with respect to statistical coverage. These methods will only strictly fulfil scaling criteria if the mechanical confinement becomes smaller than the light wavelength.

Mass spectrometry

Significant progress has been made in the development of miniaturized mass spectrometers in a variety of field applications ranging from assessing the composition of planetary atmospheres and monitoring air quality on manned space missions[36] to chemical analysis in unmanned underwater vehicles[37] and the environmental analysis of air[38,39].

Plasma discharges are used as excitation sources for molecular mass-spectrometry components and adhere to one of the oldest scaling laws we know. It is a similarity approach firstly noticed in 1915 (ref. 40). The author of this paper, Townsend[40], showed that an even earlier law[41], from 1889, was a special case of a more general similarity approach that can be applied not only to breakdown in uniform fields, but also to a breakdown that depends on ionization by collision in non-uniform fields. As shown in Fig. 3, this approach can identify the smallest dimension for which a plasma discharge can be sustained under atmospheric pressure. For helium the dimension is 24 µm; in the case of argon it is 6 µm. For analytical purposes, it makes no sense to work with higher pressures. This theorem was later extended by Holm[42] to account for the maintenance of current between geometrically similar electrodes, and its relation to fundamental processes has been discussed by von Engel and Steenbeck[43]. Margenau[44] showed theoretically how the principle might be extended to high-frequency alternating fields, and this has also been verified experimentally[45,46].

Most microplasmas developed for analytical purposes have so far concentrated on gaseous samples, which limits potential applications in the field of µTAS and lab-on-a-chip[47–49]. They are not universal devices able to measure any kind of sample. The main reason for this is the difficulty in achieving adequate sample transport from the liquid to the gas phase, and in increasing the coupled electric power into the plasma without destroying the discharge housing over a certain limit. For miniaturized plasmas, the volume and discharge power is such that even small amounts of liquid can easily extinguish the discharge. For the analysis of liquid samples, microplasmas can either be coupled with different sample-injection devices or applied as plasmas that use one electrode as a liquid, or they can be ignited directly in the liquid[50]. A lot of work is going on in this area and further studies will improve performance to the level of classical analytical plasmas. If microplasmas are to be used with liquids, sample-introduction systems that generate vapours may not always be required. Applications as detectors in a variety of liquid chromatography and capillary electrophoresis modes will require further

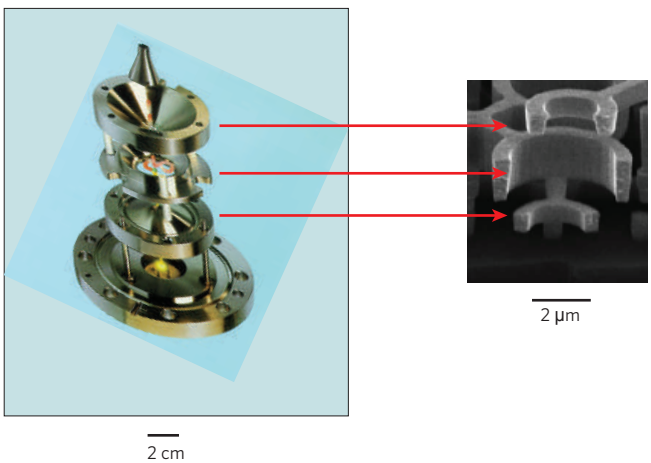


Figure 4 | Ion traps as an example of miniaturization in detection. The left panel shows the ITD700/800, a conventional ion-trap detector (image courtesy of Thermo Electron, Bremen, Germany); the right panel shows a scanning electron micrograph. The arrows indicate the two end caps and the ring electrode. Reprinted, with permission, from ref. 62.

characterization of the role of electrolyte/mobile phase identity on system performance as well. Devices such as these will have a place in the general field of elemental speciation, in which relatively simple devices of low power consumption could be widely applied.

The miniaturization of almost every type of mass analyser (quadrupole ion trap[51–53], time of flight[54–56], magnetic sector[57–59] and linear quadrupole[60,61]) is an active area of research. Miniaturized mass analysers reduce vacuum-system demands, because the maintenance of a constant collision frequency allows an increase in pressure as the analyser size is scaled down. Consequently, power consumption for vacuum and backing pumps may be reduced[36]. The quadrupole trapping technique (such as the Paul trap) is the most amenable to scaling down to the 1 μm and 10 V regime (Fig. 4). The mass-to-charge ratio of the quadrupole techniques scales proportionally to the applied voltage, inversely with the square of the characteristic length d of the analyser and the angular radio frequency, Ω. A reduction in the trap size requires that either the rf amplitude has to be decreased quadratically (for a constant Ω) or that the Ω has to be increased linearly (for a constant V) with the dimension of the trap, d.

The number of trapped charges in a 1 μm cylindrical ion trap operating at modest voltages will be small[62]. In fact, for trapping times from microseconds to milliseconds during which mass analysis can occur, it is expected that a 1 μm trap will contain at most one ion. Consequently, practical mass spectrometry with 1 μm traps will require a massive array of traps operating in parallel to obtain a useful ion signal.

The future

There has been much interest in the concept of nanofluidics and there seems to be a tremendous potential for more basic research on the subject. However, such devices fall outside the scope of many of the scaling principles described here, and new laws will have to be fully characterized. We believe that nanofluidics has not yet fully found its role. It is often said that there is plenty of room at the bottom, but perhaps not for nanofluidics in the field of traditional analytical chemistry, and not yet for commercialization. Nevertheless, inspired by the nanostructured, biochemically powered machinery of cells, a wealth of possibilities for nanoreactors and transport systems might evolve over the next decade or two.

We have already covered some specific opportunities for future research in the sections above. More broadly, the issue of integration will challenge the chemical engineer: process monitoring and control to rapidly acquire information in combination with additional reaction and preparation steps in one device.

Future research will also need to address some limitations of scaling that have so far prevented the miniaturization of certain devices. An example is high-performance liquid chromatography. Because of pressure drop, today's ultrafast high-performance liquid chromatography techniques demand pumping power of up to 400 bar. Further miniaturization would increase the pressure needed up to thousands of bar. It would not only be hard to construct such powerful pumps, but would also go beyond the mechanical stability of the chip material itself. Therefore, other ways have to be found to move the liquid phase alongside the stationary one. Besides the use of electro-osmotic flow[63], Desmet and Baron proposed the use of shear forces and demonstrated the success of this approach for straight channels[64,65].

The design of μTAS has required the use of a scientific literature spanning 200 years in a range of fields. This implies that a list of all areas of technology and applications can be consulted to find more missing links. We think that everyone could look at the literature and come up with new ideas for how to develop new approaches to designing miniaturized analysis systems. More approaches are out there, and we will continue to look for them and to exploit them in designing new miniaturized analysis systems to make laboratories more efficient and cost-effective. ■

1. Manz, A., Grabner, N. & Widmer, H. M. Miniaturized total chemical analysis systems: a novel concept for chemical sensing. *Sens. Actuators B Chem.* **1,** 244–248 (1990).
2. Golay, M. J. E. Vapor phase chromatography and the telegrapher's equation. *Anal. Chem.* **29,** 928–932 (1957).
3. van Deemter, J. J., Zuiderweg, F. J. & Klinkenberg, A. Longitudinal diffusion and resistance to mass transfer as causes of nonideality in chromatography. *Chem. Eng. Sci.* **5,** 271–289 (1956).
4. Fulford, G. D. & Catchpole, J. P. Dimensionless groups. *Ind. Eng. Chem.* **60,** 71 (1968).
5. Knox, J. H. & Gilbert, M. T. Kinetic optimization of straight open-tubular liquid-chromatography. *J. Chromatogr.* **186,** 405–418 (1997).
6. Reynolds, O. An experimental investigation of the circumstances which determine whether the motion of water shall be direct or sinuous, and of the law of resistance in parallel channels. *Phil. Trans. R. Soc. Lond.* **174,** 935–982 (1883).
7. Drexler, K. E. *Nanosystems: Molecular Machinery, Manufacturing and Computation* (John Wiley & Sons, New York, 1992).
8. Trimmer, W. S. N. Microrobots and micromechanical systems. *Sens. Actuators* **19,** 267–287 (1989).
9. Manz, A. *et al.* Planar chips technology for miniaturization and integration of separation techniques into monitoring systems. *J. Chromatogr.* **593,** 253–258 (1992).
10. Harrison, D. J. *et al.* Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. *Science* **261,** 895–897 (1993).
11. Backhouse, C. J., Gajdal, A., Pilarski, L. M. & Crabtree, H. J. Improved resolution with microchip-based enhanced field inversion electrophoresis. *Electrophoresis* **24,** 1777–1786 (2003).
12. Ludwig, M., Kohler, F. & Belder, D. High-speed chiral separations on a microchip with UV detection. *Electrophoresis* **24,** 3233–3238 (2003).
13. Xu, Y., Zhang, C.-X., Janasek, D. & Manz, A. Sub-second isoelectric focussing in free flow using a microfluidic device. *Lab Chip* **3,** 224–227 (2003).
14. Mazereeuw, M., de Best, C. M., Tjaden, U. R., Irth, H. & van der Greef, J. Free flow electrophoresis device for continuous on-line separation in analytical systems. An application in biochemical detection. *Anal. Chem.* **72,** 3881–3886 (2000).
15. Kobayashi, H. *et al.* Free-flow electrophoresis in a microfabricated chamber with a micromodule fraction separator — continuous separation of proteins. *J. Chromatogr. A* **990,** 169–178 (2003).
16. Zhang, C.-X. & Manz, A. High-speed free-flow electrophoresis on chip. *Anal. Chem.* **75,** 5759–5766 (2003).
17. Fonslow, B. R. & Bowser, M. T. Free-flow electrophoresis on an anodic bonded glass microchip. *Anal. Chem.* **77,** 5706–5710 (2005).
18. Kohlheyer, D., Besselink, G. A. J., Schlautmann, S. & Schasfoort, R. B. M. Free-flow zone electrophoresis and isoelectric focusing using a microfabricated glass device with ion permeable membranes. *Lab Chip* **6,** 374–380 (2006).
19. Albrecht, J., Gaudet, S. & Jensen, K. F. in *Ninth International Conference on Miniaturized Systems for Chemistry and Life Sciences* (eds Jensen, C. F., Han, J., Harrison, D. J. & Voldman, J.) 1537–1539 (Transducer Research Foundation, Boston, Massachusetts, 2005).
20. Janasek, D., Schilling, M., Franzke, J. & Manz, A. Isotachophoresis in free-flow using a miniaturized device. *Anal. Chem.* **78,** 3815–3819 (2006).
21. Tas, N. R., Mela, P., Kramer, T., Berenschot, J. W. & van den Berg, A. Capillary induced negative pressure of water plugs in nanochannels. *Nano Lett.* **3,** 1537–1540 (2003).
22. Eijkel, J. C. T. & van den Berg, A. Nanofluidics: what is it and what can we expect from it? *Microfluid. Nanofluid.* **1,** 249–267 (2005).
23. Eijkel, J. C. T. & van den Berg, A. The promise of nanotechnology for separation devices — from a top-down approach to nature-inspired separation devices. *Electrophoresis* **27,** 677–685 (2006).
24. Bessoth, F. G., deMello, A. J. & Manz, A. Microstructure for efficient continuous flow mixing. *Anal. Commun.* **36,** 213–215 (1999).
25. Pollack, L. *et al.* Compactness of the denatured state of a fast-folding protein measured by submillisecond small-angle X-ray scattering. *Proc. Natl Acad. Sci. USA* **96,** 10115–10117 (1999).
26. Stroock, A. D. *et al.* Chaotic mixer for microchannels. *Science* **295,** 647–651 (2002).
27. Song, H., Bringer, M. R., Tice, J. D., Gerdts, C. J. & Ismagilov, R. F. Experimental test of scaling of mixing by chaotic advection in droplets moving through microfluidic channels. *Appl. Phys. Lett.* **83,** 4664–4666 (2003).
28. Lee, C.-C. *et al.* Multistep synthesis of a radiolabeled imaging probe using integrated microfluidics. *Science* **310,** 1793–1796 (2005).
29. Lipshutz, R. J., Fodor, S. P. A., Gingeras, T. R. & Lockhart, D. J. High density synthetic oligonucleotide arrays. *Nature Genet.* **21,** 20–24 (1999).
30. Fodor S. P. A. *et al.* Light-directed, spatially addressable parallel chemical synthesis. *Science* **251,** 767–773 (1991).
31. Dittrich, P. S. & Manz, A. Single-molecule fluorescence detection in microfluidic channels — the Holy Grail in μTAS? *Anal. Bioanal. Chem.* **382,** 1771–1782 (2005).
32. Lakowitz, J. R. (ed.) *Principles of Fluorescence Spectroscopy* (Kluwer, New York, 1999).
33. Kitamori, T., Tokeshi, M., Hibara, A. & Sato, K. Thermal lens microscopy and microchip chemistry. *Anal. Chem.* **76,** 52A–60A (2004).
34. de Mello, A. J. Seeing single molecules. *Lab Chip* **3,** 29N–34N (2003).
35. Keller, R. A. *et al.* Analytical applications of single-molecule detection. *Anal. Chem.* **7,** 316A–324A (2002).
36. Palmer, P. T. & Limero, T. F. Mass spectrometry in the US space program: past, present, and future. *J. Am. Soc. Mass Spectrom.* **12,** 656–675 (2001).
37. Short, R. T., Fries, D. P., Koler, S. K., Lembke, C. E. & Byrne, R. H. Development of an underwater mass-spectrometry system for *in situ* chemical analysis. *Meas. Sci. Technol.* **10,** 1195–1201 (1999).
38. Patterson, G. E. *et al.* Miniature cylindrical ion trap mass spectrometer. *Anal. Chem.* **74,** 6145–6153 (2002).
39. Riter, L. S. *et al.* Analytical performance of a miniature cylindrical ion trap mass spectrometer. *Anal. Chem.* **74,** 6154–6162 (2002).
40. Townsend, J. S. *Electricity in Gases* 365 (Clarendon, Oxford, 1915).
41. Paschen, F. Über die zum Funkenübergang in Luft, Wasserstoff und Kohlensäure bei verschiedenen Drücken erforderliche Potentialdifferenz. *Wied. Anal. Phys. Chem.* **37,** 69–96 (1889).
42. Holm, R. Der gegenwärtige Stand der Theorie des Glimmstroms. *Phys. Z.* **25,** 497–535 (1924).
43. von Engel, A. & Steenbeck, M. *Elektrische Gasentladungen* Vol. II (Springer, Berlin, 1934).
44. Margenau, H. Theory of high frequency gas discharges. IV. Note on the similarity principle. *Phys. Rev.* **73,** 326–328 (1948).

45. Llewllyn Jones, F. & Morgan, G. D. High-frequency discharges. I. Breakdown mechanism and similarity relationship. *Proc. Phys. Soc. Lond. B* **64,** 560–573 (1951).
46. Encyclopedia of Physics Vol. XXII *Gas Discharges II* (ed. Flügge, S.) 1–40 (Springer, Berlin, 1956).
47. Franzke, J., Kunze, K., Miclea, M. & Niemax, K. Microplasmas for analytical spectrometry. *J. Anal. Atom. Spectrom.* **18,** 802–807 (2003).
48. Miclea, M., Kunze, K., Franzke, J. & Niemax, K. Microplasma jet mass spectrometry of halogenated organic compounds. *J. Anal. Atom. Spectrom.* **19,** 990–994 (2004).
49. Franzke, J. & Miclea, M. Sample analysis with miniaturized plasmas. *Appl. Spectrosc.* **60,** 80A–90A (2006).
50. Jenkins, G., Franzke, J. & Manz, A. Direct optical emission spectroscopy of liquid analytes using an electrolyte as a cathode discharge source (ELCAD) integrated on a micro-fluidic chip. *Lab Chip* **5,** 711–718 (2005).
51. Riter, L. S., Laughlin, B. C., Nikolaev, E. & Cooks, R. G. Direct analysis of volatile organic compounds in human breath using a miniaturized cylindrical ion trap mass spectrometer with a membrane inlet. *Rapid Commun. Mass Spectrom.* **16,** 2370–2373 (2002).
52. Badman, E. R. & Cooks, R. G. Cylindrical ion trap array with mass selection by variation in trap dimensions. *Anal. Chem.* **72,** 5079–5086 (2000).
53. Moxom, J., Reilly, P. T. A., Whitten, W. B. & Ramsey, J. M. Analysis of volatile organic compounds in air with a micro ion trap mass analyzer. *Anal. Chem.* **75,** 3739–3743 (2003).
54. Berkout, V. D., Cotter, R. J. & Segers, D. P. Miniaturized EI/Q/oa TOF mass spectrometer. *J. Am. Soc. Mass Spectrom.* **12,** 641–647 (2001).
55. Prieto, M. C., Kovtoun, V. V. & Cotter, R. J. Miniaturized linear time-of-flight mass spectrometer with pulsed extraction. *J. Mass Spectrom.* **37,** 1158–1162 (2002).
56. Cornish, T. J., Ecelberger, S. & Brinckerhoff, W. Miniature time-of-flight mass spectrometer using a flexible circuitboard reflector. *Rapid Commun. Mass Spectrom.* **14,** 2408–2411 (2000).
57. Diaz, J. A., Giese, C. F. & Gentry, W. R. Sub-miniature ExB sector-field mass spectrometer. *J. Am. Soc. Mass Spectrom.* **12,** 619–632 (2001).
58. Diaz, J. A., Giese, C. F. & Gentry W. R. Portable double-focusing mass-spectrometer system for field gas monitoring. *Field Anal. Chem. Technol.* **5,**156–167 (2001).
59. Diaz, J. A., Giese, C. F. & Gentry, W. R. Mass spectrometry for *in-situ* volcanic gas monitoring. *Trends Anal. Chem.* **21,** 498–514 (2002).
60. Boumsellek, S. & Ferran, R. J. Trade-offs in miniature quadrupole designs. *J. Am. Soc. Mass Spectrom.* **12,** 633–640 (2001).
61. Orient, O. J. & Chutjian, A. A compact, high-resolution Paul ion trap mass spectrometer with electron-impact ionization. *Rev. Sci. Instrum.* **73,** 2157–2160 (2002).
62. Blain, M. G. *et al.* Towards the hand-held mass spectrometer: design considerations, simulations, and fabrication of micrometer-scaled cylindrical ion trap. *Int. J. Mass Spectrom.* **236,** 91–104 (2004).
63. Bruin, G. J. M, Tock, P. P. H., Kraak, J. C. & Poppe, H. Electrically driven open-tubular liquid chromatography. *J. Chromatogr.* **517,** 557–573 (1990).
64. Desmet, G. & Baron, G. V. On the possibility of shear-driven chromatography: a theoretical performance analysis. *J. Chromatogr. A* **855,** 57–70 (1999).
65. Desmet, G. & Baron, G. V. The possibility of generating high-speed shear-driven flows and their potential application in liquid chromatography. *Anal. Chem.* **72,** 2160–2165 (2000).
66. de la Rue, W. & Muller, H. W., Experimental researches on the electric discharge with the chloride of silver battery. *Phil. Trans. R. Soc. Lond.* **171,** 65–116 (1880).
67. Carr, W. R. On the laws governing electric discharges in gases at low pressures. *Phil. Trans. R. Soc. Lond. A* **201,** 403–433 (1903).

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