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REVIEW

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Airborne chemistry: acoustic levitation in chemical analysis

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Abstract This review with 60 references describes a unique path to miniaturisation, that is, the use of acoustic levitation in analytical and bioanalytical chemistry applications. Levitation of small volumes of sample by means of a levitation technique can be used as a way to avoid solid walls around the sample, thus circumventing the main problem of miniaturisation, the unfavourable surface-to-volume ratio. Different techniques for sample levitation have been developed and improved. Of the levitation techniques described, acoustic or ultrasonic levitation fulfils all requirements for analytical chemistry applications. This technique has previously been used to study properties of molten materials and the equilibrium shapeand stability of liquid drops. Temperature and mass transfer in levitated drops have also been described, as have crystallisation and microgravity applications.

The *airborne analytical system* described here is equipped with different and exchangeable remote detection systems. The levitated drops are normally in the 100 nL–2 μL volume range and additions to the levitated drop can be made in the pL-volume range.

The use of levitated drops in analytical and bioanalytical chemistry offers several benefits. Several remote detection systems are compatible with acoustic levitation, including fluorescence imaging detection, right angle light scattering, Raman spectroscopy, and X-ray diffraction. Applications include liquid/liquid extractions, solvent exchange, analyte enrichment, single-cell analysis, cell–cell communication studies, precipitation screening of proteins to establish nucleation conditions, and crystallisation of proteins and pharmaceuticals.

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Introduction

Advances in understanding how cells differentiate, function and interact are vital in the search for new therapeutic targets and pharmaceuticals. The "omics" research areas, comprising genomics, proteomics, crystallomics, organellomics, metabolomics, and cellomics, aim at defining the status of every gene, protein, organelle, and metabolic profile in a given cell under different conditions. Due to the growing number of "omics" research projects, development of user-friendly, sensitive and biocompatible miniaturisation technologies is of the utmost concern for applications in biology, biomedicine, biotechnology and chemical and pharmaceutical analysis. There is now a lack of analytical systems combining high sample throughput with the ability to extract high-quality information from biological and biochemical events. One main quality of such a system would be miniaturisation in order to keep sample and chemical consumption low. This is imperative if sample access is limited (e.g. in single-cell analysis and early analysis of drug candidates). Chip-based analytical systems have partly achieved this goal and mostly involve electrophoretically driven flows, that is electrophoresis and electrochromatography [1, 2, 3]. There have been a number of recent advances in the fields of miniaturised reaction and separation systems, including the construction of fully integrated 'lab-on-a-chip' systems [4, 5]. Many techniques for studying dynamic events at the single-cell level have been developed, including fluorescence imaging techniques [6, 7], capillary electrophoresis [8, 9], flow cytometry [2] and capillary liquid chromatography [10].

However successful in certain applications, miniaturisation and single-cell studies in such systems have their own difficulties [11]. Among the most serious drawbacks is the risk of analyte adsorption to walls and interfaces [12, 13, 14, 15, 16, 17], a problem multiplied in small-dimensional analytic systems where volumes in the picolitre (pL) or nanolitre (nL) range are used. Another problem is optical interference at the walls of the sample container hampering detection [15], especially since such low amounts of analytes are being used. Therefore, future advances in these areas require the development of analytical techniques capable of circumvent-

ing the use of contacting walls and thereby avoiding these problems and yet able to monitor minute cellular changes induced by ligands, drugs, activators, or inhibitors.

Levitation techniques

A number of physical effects allow free floatation, levitation, of solid and liquid matter. Levitation of small volumes of sample by means of a levitation technique can be used as a way to avoid solid walls around the sample. The only contacting surface is thus the surrounding medium, which is commonly air. The use of levitated drops exhibits the same miniaturisation benefits as the chip approach, such as diversity of application and low reagent and sample consumption. In addition to the obvious advantage of levitation for preventing the chemical and thermal contamination that accompanies contact between drops and external objects, the use of levitated drops also has the added advantage of increased sensitivity of detection, since no walls disturb detection [12, 15].

Different techniques for sample levitation have previously been developed and improved [18]. These techniques include optical levitation [19], electrostatic levitation [20], aerodynamic levitation [18], diamagnetic levitation [21] and acoustic levitation [12].

However, for a levitation technique to be useful as a general, bioanalytical technique, it must combine biological compatibility with ease of sample handling such as stable sample position, easy access to the sample, a wide range of sample volumes and low costs for supply and operation [12]. In contrast to the other levitation techniques, acoustic levitation also has the advantage of not requiring any specific physical properties (e.g. electric charge or a certain refractive index) of the sample. Consequently almost all substances whether they are solids or liquids can be acoustically levitated.

Optical levitation

Optical levitation [22] is achieved by using a high numerical aperture lens to sharply focus a laser beam. By illuminating an object by such a focused laser beam, the object is subjected to a scattering force that pushes it forward into the direction of the energy flux. A gradient force is also exerted to pull the object towards regions with high electrical density (generally the focal region). In regions where the two forces cancel each other particles may be trapped at a stationary three-dimensional (3D) position [23]. Examples of applications are 3D cellular and intracellular micromanipulation [24, 25] and cell sorting [26].

Diamagnetic levitation

Levitation can be achieved without the need for energy input by the use of diamagnetism. Diamagnetism refers to the ability of a material to expel a portion of an external magnetic field. Electrons in such materials rearrange their orbits slightly so that they expel the external field. As a result, diamagnetic materials repel and are repelled by strong magnetic fields. Superconductors are ideal diamagnetics and completely expel magnetic field at low temperatures. But an object does not need to be superconducting to levitate if placed in a strong enough magnetic field [27]. Use of magnetic field strengths of about 10 Tesla has been described, and water droplets and even frogs have been levitated in this way at Nijmegen Magnetics Laboratory in the Netherlands [21].

Electrostatic levitation

In an electrostatic levitation system, a charged drop is levitated by applying electrostatic fields from a set of properly arranged electrodes. The sample is positioned by actively controlling an applied electrostatic field. A large levitation force is required to compensate for the gravitational pull, which in practice means large sample charges and a strong applied electric field (or large potential difference between electrodes). The requirement for a high-voltage output can make this approach somewhat inconvenient. Electrostatic levitation has, among other things, found use in protein crystal growth [28] and X-ray scattering studies of metallic liquids [29].

Aerodynamic levitation

Aerodynamic levitation is achieved by lifting spherical specimens by a fluid jet. The divergence of the jet leads to a decreasing drag with increasing height, which gives stability in the vertical direction. In the transverse direction stable levitation is achieved because the jet is deflected towards an off-axis specimen. Aerodynamic levitation has found much use in industrial applications and for levitating solid spheres. Vibrational instabilities have been described for aerodynamic levitation of liquids [22].

Acoustic levitation

The phenomenon that small samples can be levitated in the nodal points of a standing ultrasonic wave was first described in 1933 [30]. The theory behind this phenomenon is well understood [22, 31]. A standing wave with equally spaced nodes and antinodes is created by multiple reflections between an ultrasonic radiator and a solid reflector. Figure 1 shows a levitated water drop (i.e. "wall-less" test tube) positioned in such a nodal point between the ultrasonic transducer and the reflector.

Acoustic levitation has been used to study properties of molten materials and the equilibrium shape [32] and stability of liquid drops. The technique has been employed for density measurements [33] and in the study of surface-tension-dominated fluid dynamic phenomena with applications in microgravity [27, 28]. Streaming flows associ-

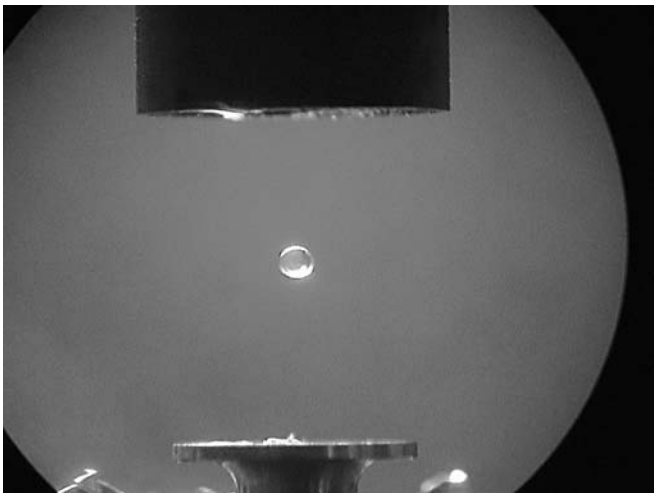

Fig. 1 A 500-nLwater drop levitated in a node in a standing wave created between an ultrasonic transducer (*bottom*) and a solid reflector (*top*)

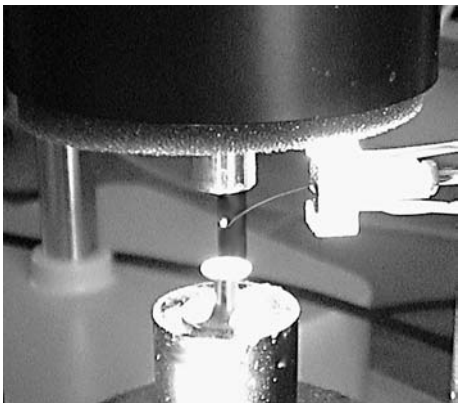

Fig. 2 Dispenser droplet addition to the levitated drop. The dispenser trajectory is seen as the *thin white line* connecting the dispenser nozzle on the *right* with the levitated drop

Fig. 3 Two-phase (aq./org.) system in levitated drop. The aqueous phase is in *yellow* and the organic phase in *orange*

ated with ultrasonic levitators [34] and effects of acoustic streaming [35] have been discussed, as have flowfield characteristics of aerodynamic acoustic levitators [36] and effects of acoustic streaming [35]. Temperature and mass transfer in levitated drops have also been described [32, 33].

Extensive studies have also been performed on evaporation [37, 38] and drying [39] of acoustically levitated droplets.

Airborne chemistry

Applications

Acoustic levitation has begun to attract interest for use in analytical and bioanalytical chemistry applications. In 1982, Apfel and co-workers discussed the development of acoustic levitation for measuring the mechanical properties, namely density, compressibility, and sound velocity, of biological materials [40]. Later, Neidhart and co-workers reported their first experiences with the technique of acoustical levitation of droplets in the field of analytical and atmospheric chemistry [12]. They performed the following common experiments of sample preparation procedures in levitated drops: acid–base titrations, liquid/liquid extractions, solvent exchange and analyte enrichment by evaporation of the solvent. They also reported the formation and growth of ice particles and trapping of heavy gases [41] in stationary ultrasonic fields as well as sample preparation prior to gas chromatography [12]. Klockow and co-workers have performed titrations and crystallisation experiments [42] and investigated phase transfer and freezing processes on acoustically levitated aqueous droplets [43].

A basic analytical system (Fig. 2) consisting of an acoustic levitator combined with flow-through pL dispensers [14, 44, 45] has been used in our laboratory for bioanalytical purposes. This *airborne analytical system* [15, 39, 40, 41, 42] is equipped with different and exchangeable remote detection systems. The levitated drops are normally in the 100 nL–2 µL volume range and additions to the levitated drop can be made in the pL-volume range. It has been shown that the airborne system can be successfully used for single-cell analysis, [15, 39] crystallisation studies of pharmaceuticals [46] and proteins [47] as well as for sample enrichment prior to capillary electrophoresis/capillary electrochromatography (CE/CEC) analysis [14].

For the single-cell experiments, a buffer drop containing living cells is levitated and different substances are presented to the cells using the pL flow-through dispensers. The cell response (or lack of response) is then monitored using fluorescence imaging detection. In our laboratory, methods for liquid/liquid cell organelle extractions in levitated drops are presently being developed. Figure 3 shows an aqueous/organic two-phase system in a levitated drop. This approach will allow the study at the single-cell level of the up-and-down regulation of intracellular molecules in response to environmental factors such as other cells, substances produced by other cells, or drugs. In the levitated drop, cells are first stimulated/inhibited and then lysed by addition of an organic solvent. The organic solvent also creates a two-phase system into which the cells release their intracellular molecules that are extracted to one of the phases. The use of affinity aqueous two-phase systems, currently under development in our laboratory, would further allow isolation of subcellular fractions from single cells by affinity two-phase partitioning.

Levitation has also attracted substantial interest for protein crystallisation, especially with respect to microgravity applications. Ishikawa and Komada have discussed the development of acoustic and electrostatic levitators for containerless protein crystallization [48]. Rhim and Chung used an electrostatic multi-drop levitation system [49] and Chung and Trinh used an ultrasonic–electrostatic hybrid levitator for growing protein crystals from levitated drops with the aim to create controlled crystal growth conditions which would reproduce some of the aspects of a low-gravity environment [28]. In our laboratory, a screening method based on acoustically levitated drops was developed for the study of precipitation of proteins for crystallisation purposes [47]. At present, crystals are mostly grown using trial-and-error procedures, and protocols that rapidly screen for the crystal nucleation step are rare. Our approach is to minimise the consumption of protein material while searching for the nucleation conditions in a rational manner. Levitated drops of known protein concentration are injected with crystallising agents using piezoelectric flow-through dispensers and calculations are performed giving the concentrations of all components in the drop at any time during an experiment. Protein precipitation is monitored using right-angle light scattering. Precipitation diagrams are then constructed giving the protein/crystallising agent concentration boundaries between the minimum and the maximum detectable protein precipitation. This approach is especially suitable if previous screening for nucleation conditions of a protein has failed [47]. Recently, some interesting microfluidic approaches to screening of protein nucleation conditions have been described [50], which utilize even smaller volumes than the levitated approach developed in our laboratory. However, the authors claim that their system is especially suited for the optimisation step in crystallization. These two approaches, levitation and microfluidic, could therefore be complementary in the search for protein nucleation conditions.

Sample handling

Accurate free hand positioning of an acoustically levitated drop requires some practice. Basically any kind of syringe, pipette or capillary may be used for drop positioning in the ultrasonic field [12], provided that small enough drops can be produced. To aid drop detachment from the tip, the tip can be coated with a hydrophobic substance. Difficulties in drop detachment also increase with decreased surface tension and/or viscosity of the liquid. Operating at increased ultrasonic power during the positioning also enhances drop detachment. After positioning the levitated drop may perform oscillations, but it can be readily stabilised by adjusting the distance between the transducer and the reflector and by setting the ultrasonic power properly [14, 15].

Sample delivery can also be achieved by using a non-contact delivery device (e.g. some variety of ink-jet printing technology which allows droplets to fly freely over a distance). The volumetric accuracy of ink-jet dispensing is not affected by how the fluid wets a substrate and the fluid source cannot be contaminated by the substrate. One example of such a device is the flow-through droplet dispenser used in our laboratory, which was developed in-house at the department of Electrical Measurements, Lund University, Sweden. The main advantages of the flow-through dispenser are the possibility to dispense samples from flowing liquids, the high-precision non-contact mode of sample supply, the small size of the droplets (pL-volume range) and the high droplet ejection frequency, up to 9 kHz [14, 51].

After analysis in the levitated drop, samples can be removed from the levitator by using the same type of syringe/micropipette/capillary used to position them there. Removing aliquots from the levitated drop is best achieved by using a very thin tip, since otherwise the levitated drop is most likely to catch on the tip and be pulled out of the node [52]. A nano-tip suitable for withdrawing aliquots from the drop has been developed in our laboratory [53]. If the whole drop is to be removed, it can either be caught on the tip and placed in a microtiter plate for observation under microscope, or introduced in for example a capillary for further analysis [52].

Detection systems

In order to truly benefit from the use of levitated drops, remote and non-invasive detection protocols are important. This can be achieved by using varieties of spectroscopy [42]. Neidhart and co-workers used a diode array spectrometer designed for use with optical fibres for absorption and fluorescence measurements [13]. A method for non-contact fluorescence thermometry of acoustically levitated water drops was developed by Seaver and Peele [54]. Fluorescence imaging detection based on previous work on real-time fluorescence imaging for capillary electrophoresis [55, 56] was also used by our group for single-cell analysis [15, 39]. The experimental set-up is shown in

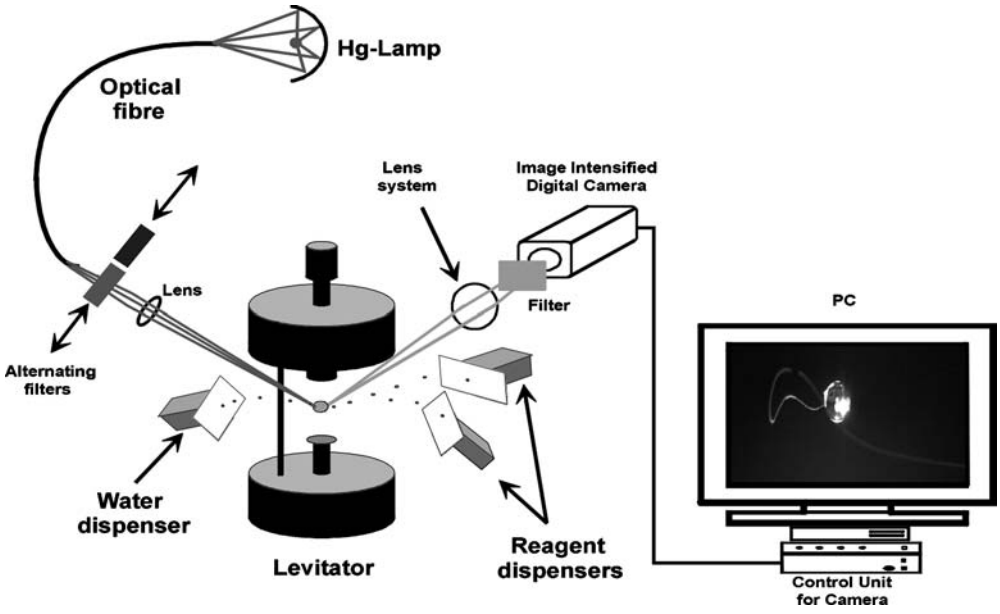

Fig. 4 Instrumental set-up for airborne cell experiments using fluorescence imaging detection consisting of a Hg lamp, an optical fibre, two alternating interference filters (405 and 435 nm), a lens, an acoustic levitator, continuous flow-through droplet dispensers, a lens system, another interference filter (510 nm), a CCD camera and a computer

Fig. 4. The main aim has been to develop a method able to detect single-cell responses from fat cells and other cell types involved in fat metabolism, thus making possible a search for differences between individual cells and cell responses in order to aid in the understanding of diabetes and related diseases.

Right-angle light-scattering detection was applied by our group to the study of precipitation of proteins for crystallisation purposes [47]. Biswas described solidification of acoustically levitated crystals using Raman spectroscopy [57]. Raman spectroscopy was also employed by our group for studies of crystallization processes in levitated drops [46]. Crystallization studies on the model compounds benzamide and indomethacin resulted in the formation of two crystal modifications for each compound, suggesting that this methodology may be useful for investigation of polymorphs. Surface-enhanced Raman scattering (SERS) detection in combination with levitated drops was demonstrated by our group [46]. Lendl and co-workers used FT-Raman spectroscopy for in situ synthesis and application of SERS-active Ag-sols for trace analysis [58]. In the future, several important applications of the combination between Raman spectroscopy and acoustic drop levitation are to be expected.

Tests have been performed by our group to evaluate the use of acoustically levitated drops for liquid X-ray diffraction [59]. The tests were performed at the crystallography beamline I711 at the MAX II synchrotron in Lund, Sweden. The results showed that a droplet of liquid and solid (powder) samples can be kept in an X-ray beam for sufficient time to allow collection of the X-ray diffraction pattern. X-ray scattering from levitated liquids has previously been extensively described for several other levitation techniques [60].

Conclusions

The use of acoustically levitated drops in analytical and bioanalytical chemistry offers several benefits. Several remote detection systems are compatible with acoustic levitation, including fluorescence imaging detection, right-angle light scattering, Raman spectroscopy, and X-ray diffraction. Applications include liquid/liquid extractions, solvent exchange, analyte enrichment, single-cell analysis, cell–cell communication studies, precipitation screening of proteins to establish nucleation conditions, and crystallisation of proteins and pharmaceuticals. The diversity of applications possible, the biological compatibility, the small volumes, and the absence of contacting surfaces are all important features and strongly support the further use and development of this new approach in bioanalytical chemistry. A growing area of science utilising levitation systems can be foreseen, especially in the different "omics" research areas where high-quality information is necessary.

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References

1. Bruin GJM (2000) Electrophoresis 21:3931–3951
2. McClain MA, Culbertson CT, Jacobson SC, Ramsey JM (2001) Anal Chem 73:5334–5338
3. Li JJ, Thibault P, Bings NH, Skinner CD, Wang C, Colyer C, Harrison J (1999) Anal Chem 71:3036–3045
4. Krishnan M, Namasivayam V, Lin RS, Pal R, Burns MA (2001) Curr Opin Biotech 12:92–98
5. Manz A, Eijkel JCT (2001) Pure Appl Chem 73:1555–1561
6. Yeung ES (1999) Anal Chem 71:A522–A529
7. Tong W, Yeung ES (1998) Appl Spectrosc 52:407–413
8. Krylov SN, Arriaga EA, Chan NWC, Dovichi NJ, Palcic MM (2000) Anal Biochem 283
9. de Mello AJ (2003) Lab Chip 3:29N–34N
10. Chen G, Ewing AG (1997) Crit Rev Neurobiol 11:59–90
11. Cannon DM, Winograd N, Ewing AG (2000) Annu Rev Bioph Biom 29:239–263
12. Welter E, Neidhart B (1997) Fresenius J Anal Chem 357:345–350
13. Rohling O, Weitkamp C, Neidhart B (2000) Fresenius J Anal Chem 368:125–129
14. Petersson M, Nilsson J, Wallman L, Laurell T, Johansson J, Nilsson S (1998) J Chromatogr B 714:39–46
15. Santesson S, Andersson M, Degerman E, Johansson T, Nilsson J, Nilsson S (2000) Anal Chem 72:3412–3418
16. Nilsson S, Santesson S, Degerman E, Cedergren E, Johansson T, Nilsson J, Johansson J (2000) Proceedings of SPIE, scanning and force microscopies for biomedical applications II. San José, California, USA 2000, pp 81–90
17. Nilsson S, Santesson S, Degerman E, Johansson T, Laurell T, Nilsson J, Johansson J (2000) Micro total analysis systems. Kluwer, The Netherlands, pp 19–24
18. Lierke EG (1995) Forsch Ingenieurwes 61:201–216
19. Hoffmann HG, Lentz E, Schrader B (1993) Rev Sci Instrum 64:823–824
20. Barnes MD, Ng KC, Whitten WB, Ramsey JM (1993) Anal Chem 65:2360–2365
21. Berry MV, Geim AK (1997) Eur J Phys 18:307–313
22. Brandt EH (1989) Science 243:349–355
23. Nahmias YK, Odde DJ (2002) IEEE J Quantum Electron 38: 131–141
24. Visscher K, Brakenhoff GJ, Krol JJ (1993) Cytometry 14:105–114
25. Konig K (1998) Cell Mol Biol 44:721–733
26. Grover SC, Skirtach AG, Gauthier RC, Grover CP (2001) J Biomed Opt 6:14–22
27. Simon MD, Geim AK (2000) J Appl Phys 87:6200–6204
28. Chung SK, Trinh EH (1998) J Cryst Growth 194:384–397
29. Kelton KF, Lee GW, Gangopadhyay AK, Hyers RW, Rathz TJ, Rogers JR, Robinson MB, Robinson DS (2003) Phys Rev Lett 90:Art No 195504
30. Bücks K, Müller HZ (1933) Phys 84:75–86
31. Lierke EG (1996) Acustica 82:220–237
32. Trinh EH, Hsu CJ (1986) J Acoust Soc Am 79:1335–1338
33. Trinh EH, Hsu CJ (1986) J Acoust Soc Am 80:1757–1761
34. Trinh EH, Robey JL (1994) Phys Fluids 6:3567–3579
35. Kawahara N, Yarin AL, Brenn G, Kastner O, Durst F (2000) Phys Fluids 12:912–923
36. Yarin AL, Brenn G, Keller J, Pfaffenlehner M, Ryssel E, Tropea C (1997) Phys Fluids 9:3300–3314
37. Trinh EH, Marston PL, Robey JL (1998) J Colloid Interface Sci 124:95–103
38. Bayazitoglu Y, Mitchell GF (1995) J Thermophys Heat Transfer 9:694–701
39. Yarin AL, Brenn G, Kastner O, Tropea C (2002) Phys Fluids 14:2289–2298
40. Weiser MH, Apfel RE (1982) J Acoust Soc Am 71:1261–1268
41. Tuckermann R, Neidhart B, Lierke EG, Bauerecker S (2002) Chem Phys Lett 363:349–354
42. Davies AN, Jacob P, Stockhaus A, Kuckuk R, Hill W, Hergenroder R, Zybin A, Klockow D (2000) Appl Spectrosc 54:1831–1836
43. Jacob P, Stockhaus A, Hergenroder R, Klockow D (2001) Fresenius J Anal Chem 371:726–733
44. Yarin AL, Brenn G, Kastner O, Rensink D, Tropea C (1999) J Fluid Mech 399:151–204
45. Yarin AL, Brenn G, Rensink D (2002) Int J Heat Fluid Fl 23: 471–486
46. Santesson S, Johansson J, Taylor LS, Levander I, Fox S, Sepaniak M, Nilsson S (2003) Anal Chem 75:2177–2180
47. Santesson S, Cedergren-Zeppezauer ES, Johansson T, Laurell T, Nilsson J, Nilsson S (2003) Anal Chem 75:1733–1740
48. Ishikawa Y, Komada S (1993) FUJITSU Sci Tech J 29:330–338
49. Rhim W, Chung S (1991) J Cryst Growth 110:293–301
50. Zheng B, Roach LS, Ismagilov RF (2003) J Am Chem Soc 125:11170–11171
51. Santesson S, Johansson T, Nilsson J, Degerman E, Rorsman P, Viberg P, Spégel P, Nilsson S (2001) ASMS, Chicago
52. Santesson S (2002) Licentiate Thesis, Lund University, Lund
53. Santesson S, Barinaga-Rementeria Ramírez I, Viberg P, Jergil B, Nilsson S (2003) Anal Chem 78:303-308
54. Seaver M, Peele JR (1990) Appl Optics 29:4956–4961
55. Johansson J, Witte DT, Larsson M, Nilsson S (1996) Anal Chem 68:2766–2770
56. Johansson T, Petersson M, Johansson J, Nilsson S (1999) Anal Chem 71:4190
57. Biswas A (1995) J Cryst Growth 147:155–164
58. Leopold N, Haberkorn M, Laurell T, Nilsson J, Baena JR, Frank J, Lendl B (2003) Anal Chem 75:2166–2171
59. Cerenius Y, Oskarsson Å, Santesson S, Nilsson S, Kloo LJ (2003) Appl Crystallogr 36:163–164
60. Krishnan S, Price DL (2000) J Phys Condens Matter 12:R145–R176