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Microfoam formation in a capillary

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Abstract

The ultrasound-induced formation of bubble clusters may be of interest as a therapeutic means. If the clusters behave as one entity, *i.e.*, one mega-bubble, its ultrasonic manipulation towards a boundary is straightforward and quick. If the clusters can be forced to accumulate to a microfoam, entire vessels might be blocked on purpose using an ultrasound contrast agent and a sound source.

In this paper, we analyse how ultrasound contrast agent clusters are formed in a capillary and what happens to the clusters if sonication is continued, using continuous driving frequencies in the range 1–10 MHz. Furthermore, we show high-speed camera footage of microbubble clustering phenomena.

We observed the following stages of microfoam formation within a dense population of microbubbles before ultrasound arrival. After the sonication started, contrast microbubbles collided, forming small clusters, owing to secondary radiation forces. These clusters coalesced within the space of a quarter of the ultrasonic wavelength, owing to primary radiation forces. The resulting microfoams translated in the direction of the ultrasound field, hitting the capillary wall, also owing to primary radiation forces.

We have demonstrated that as soon as the bubble clusters are formed and as long as they are in the sound field, they behave as one entity. At our acoustic settings, it takes seconds to force the bubble clusters to positions approximately a quarter wavelength apart. It also just takes seconds to drive the clusters towards the capillary wall.

Subjecting an ultrasound contrast agent of given concentration to a continuous low-amplitude signal makes it cluster to a microfoam of known position and known size, allowing for sonic manipulation.

Key words: Capillary blocking, Embolisation, Microfoam, Radiation forces, Ultrasound contrast agent

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1 Introduction

2 Ultrasound contrast agents are used in diagnostic imaging. They consist of
3 microscopically small bubbles containing slowly diffusing gas encapsulated
4 by biodegradable shells. When inserted in the blood stream, these bubbles
5 oscillate upon ultrasonic sonication, thereby creating detectable ultrasound
6 themselves. A brief overview of the most common ultrasound contrast agents
7 has been presented in [1]. It follows that albumin and lipids are currently
8 the most common bubble encapsulation materials. Because of the proven
9 feasibility to attach therapeutic compounds to albumin and lipids, therapeutic
10 applications of contrast agents have become of interest [2–5]. It is desirable that
11 the therapeutic load of any such contrast agent is released close to the vessel
12 wall. Therefore, pushing bubbles towards boundaries by means of primary
13 radiation forces has been studied [6]. Both primary and secondary radiation
14 forces resulting from oscillating bubbles, may cause the repulsion or mutual
15 attraction, and eventual collision and coalescence, of contrast agent bubbles.
16 This phenomenon has been less studied.

17 From the therapeutic point of view, the formation of bubble clusters may
18 be of interest. If the clusters behave as one entity, *i.e.*, one mega-bubble,
19 its ultrasonic manipulation towards a boundary is fairly straightforward and
20 quick. If the clusters can be forced to accumulate to a microfoam, entire vessels
21 might be blocked on purpose using an ultrasound contrast agent and a sound
22 source.

23 In this paper, we analyse how ultrasound contrast agent clusters are formed
24 and what happens to the clusters if sonication is continued. Furthermore, we
25 show high-speed camera footage of microbubble clustering phenomena and
26 discuss the therapeutic consequences of our findings.

27 **2 Theory**

28 A brief overview of theory on radiation forces and ultrasound contrast agent
 29 has been given in [7]. Bubble translation in the direction of the sound field is
 30 caused by a primary radiation force resulting from a pressure gradient across
 31 the bubble surface. The translation is maximal in contraction phase. The
 32 velocity v of a bubble in a steady fluid subjected to an ultrasound field can
 33 be calculated using [8]:

$$34 \quad F_r + F_d - \frac{d(mv)}{dt} \approx 0, \quad (1)$$

35 where F_r is the primary radiation force, F_d is the drag force, $m = \frac{2}{3}\pi\rho R_0^3$ is
 36 the added mass of the translating bubble, equivalent to half the mass of the
 37 displaced surrounding fluid, in which R_0 is the equilibrium bubble radius and
 38 ρ is the density of the surrounding fluid. Averaging over one acoustic cycle,
 39 the primary radiation force is given by [8,9]:

$$40 \quad F_r = \frac{p_a^2 R_0}{\rho c f} \frac{\delta \left(\frac{f_0}{f} \right)}{\left[\left(\frac{f_0}{f} \right)^2 - 1 \right]^2 + \left[\delta \left(\frac{f_0}{f} \right) \right]^2}, \quad (2)$$

41 where c is the speed of sound, p_a is the peak rarefactional acoustic pressure, δ
 42 is the dimensionless total damping coefficient [10], f is the driving frequency,
 43 and f_0 is the bubble resonance frequency [10]. The drag force is given by [9,11]:

$$44 \quad F_d = -\frac{\pi\eta}{4} C_d \text{Re} R_0 v(t), \quad (3)$$

45 where η is the shear (dynamic) viscosity of the fluid, $\text{Re} = \frac{2\rho R_0}{\eta} |v(t)|$ is the
 46 Reynolds number, and

$$47 \quad C_d = \frac{24}{\text{Re}} (1 + 0.15 \text{Re}^{0.687}) \quad (4)$$

48 is the drag coefficient of a contaminated system [12], such as a contrast agent.

49 Combining equations (1)–(3) and integrating over dt gives the following
 50 expression for the average velocity of a bubble:

$$51 \quad v = \frac{4p_a^2}{\rho c f \eta C_d \text{Re}} \frac{\delta \left(\frac{f_0}{f} \right)}{\left[\left(\frac{f_0}{f} \right)^2 - 1 \right]^2 + \left[\delta \left(\frac{f_0}{f} \right) \right]^2} \left[1 - e \left(-\frac{3 \eta C_d \text{Re}}{8 \rho R_0^2} t \right) \right]. \quad (5)$$

52 Secondary radiation forces, resulting from oscillating bubbles under sonication,
 53 may cause the mutual attraction and subsequent coalescence of contrast
 54 microbubbles. Two bubbles that oscillate in phase approach each other,
 55 whereas two bubbles that oscillate out of phase recede from each other [13,14].
 56 At low acoustic amplitudes, the phase angle difference ϕ between excursion of
 57 the oscillating bubble and the incident sound field is given by [13,14]:

$$58 \quad \phi = \pi + \arctan \left(\frac{\delta \left(\frac{f}{f_0} \right)}{1 - \left(\frac{f}{f_0} \right)^2} \right). \quad (6)$$

59 The presence of an encapsulating shell increases the damping coefficient by a
 60 term δ_s [15]

$$61 \quad \delta_s = \frac{S_f}{2\pi m f_0}, \quad (7)$$

62 and increases the squared resonance frequency f_0^2 by a term f_s^2 [15]

$$63 \quad f_s^2 = \frac{\chi}{2\pi R_0^3 \rho}, \quad (8)$$

64 where S_f is the shell friction [15] and χ is the shell stiffness parameter [14,15]

$$65 \quad \chi = \frac{E\epsilon}{1-\nu}, \quad (9)$$

66 in which E is Youngs modulus, ϵ is the shell thickness, and ν is the Poisson
 67 ratio.

68 The mean approach velocity u of two identical bubbles is given by [8]:

$$69 \quad u = \frac{dd}{dt} = -\frac{(2\pi f p_a)^2}{27 \eta} \rho \kappa^2 \frac{R_0^5}{d^2}, \quad (10)$$

70 where d is the distance between the centres of the two bubbles and κ is the
 71 compressibility of the bubble. Integrating from the initial distance between
 72 the bubbles d_0 to 0 yields the collision time

$$73 \quad t_c = - \int_{d_0}^0 \frac{27\eta}{(2\pi f p_a)^2 \rho \kappa^2 R_0^5} d^2 dd = \frac{9\eta}{(2\pi f p_a)^2 \rho \kappa^2 R_0^5} d_0^3. \quad (11)$$

74 In a standing wave field, bubbles with resonance frequencies higher than the
 75 transmitted sound field aggregate at the pressure antinodes, whereas bubbles
 76 with resonance frequencies lower than the transmitted sound field aggregate
 77 at the pressure nodes [13]. Hence, the ultimate distance d_∞ between clusters
 78 must be a quarter of the wavelength, *i.e.*,

$$79 \quad d_\infty = \frac{\lambda}{4} = \frac{c}{4f}. \quad (12)$$

80 Both processes of bubble clusters aggregating and the movement of clusters
 81 in the direction of the sound field can be described by a simplified version of
 82 (5):

$$83 \quad v = \frac{dh}{dt} \approx \frac{p_a^2}{6 \rho c f \eta} \frac{\delta \left(\frac{f_c}{f} \right)}{\left[\left(\frac{f_c}{f} \right)^2 - 1 \right]^2 + \left[\delta \left(\frac{f_c}{f} \right) \right]^2}, \quad (13)$$

84 where h is the distance travelled by the cluster and f_c is the cluster resonance
 85 frequency, for which $f_c < f_0$ must hold, since the bubble cluster radius
 86 $R_c > R_0$. For the bubble cluster compressibility κ_c , $\kappa \leq \kappa_c < \kappa_f$ must hold, in
 87 which κ_f is the compressibility of a free (unencapsulated) gas bubble.

88 Bubble coalescence is the fusion of two or more bubbles. As adjacent bubbles
 89 collide or expand, the pressure in the film between them increases, resulting
 90 in a deformation (flattening) of the bubble surfaces. The continuing bubble
 91 expansion causes drainage of the interposed film. This thinning continues until
 92 a critical thickness around $0.1 \mu\text{m}$ is reached, at which the Van der Waals
 93 attractive forces result in film rupture and the coalescence of the bubbles [16].
 94 Film drainage is generally much faster for free (unencapsulated) bubbles than
 95 for encapsulated bubbles, as a result of the flow pattern in the draining film
 96 [17].

97 The coalescence mechanism of lipid-encapsulated microbubbles was
 98 investigated, based on high-speed optical observations of sonicated
 99 lipid-encapsulated ultrasound contrast agent microbubbles [17]. It was

100 found that, when sonicated at high acoustic amplitudes, lipid-encapsulated
101 microbubbles expose free surfaces during the expansion phase, speeding up the
102 coalescence process dramatically. Hence, for the formation of bubble clouds or
103 microfoams, the use of low acoustic amplitudes is desirable.

104 3 Materials and Methods

105 A schematic overview of our experimental setup for simultaneous optical
106 observations during sonication is shown in Figure 1. A polycarbonate container
107 was built with internal dimensions: $24 \times 18 \times 15$ (cm)³. To give access to a
108 microscope objective lens, a hole with an 11-mm diameter had been drilled
109 in the base, covered with a 2-mm thick test slide (Jencons (Scientific) Ltd,
110 Leighton Buzzard, Bedfordshire, UK). The container was filled with 2.6 L tap
111 water. The container was placed on an x - y -table on top of a DM IRM inverted
112 microscope (Leica Microsystems Wetzlar GmbH, Wetzlar, Germany) with two
113 objective lenses: a 506075 C-Plan 10 \times objective lens (Leica Microsystems
114 Wetzlar GmbH) with a 0.22 numerical aperture and a 506236 N-Plan 50 \times
115 objective lens (Leica Microsystems Wetzlar GmbH) with a 0.50 numerical
116 aperture. A Mille LuceTM Fiber Optic Illuminator Model M1000 (StockerYale,
117 Inc., Salem, NH, USA) was connected to an optic fibre with a 7-mm diameter
118 leading into the water of the container. It was placed in line with the objective
119 lens, as shown in Figure 2.

120 The charge couple device (CCD) of a FASTCAM MC1 high-speed camera
121 (Photron (Europe) Limited, West Wycombe, Bucks, United Kingdom) was
122 mounted to the microscope and connected to its processing unit, which was
123 capable of recording images at 10,000 frames per second. The camera was
124 controlled by a laptop computer.

125 3.1 Ultrasound

126 A laptop computer triggered a DATAMAN-530 arbitrary waveform generator
127 (Dataman Programmers Ltd, Maiden Newton, Dorset, UK), which was
128 connected to a 2100L 50-dB RF power amplifier (Electronics & Innovation
129 Ltd., Rochester, NY, USA). The power amplifier was connected to an
130 undamped broadband single element transducer containing a Pz37 Piezo
131 crystal (Ferroperm Piezoceramics A/S, Kvistgård, Denmark) with a centre
132 frequency of 2.2 MHz. The design of the transducer has been described
133 in [18]. Transmitted signals were typically continuous with frequencies in
134 the range 1-10 MHz. The peak-negative acoustic pressures were determined
135 using a PVdF needle hydrophone system with a 0.2-mm probe (Precision
136 Acoustics Ltd, Dorchester, Dorset, UK) connected to a TDS 420A oscilloscope
137 (Tektronix, Inc., Beaverton, OR, USA).

138 The ultrasound transducer was positioned in the container using a clamp
139 stand, at a focal distance of 38 mm from the region of interest to be studied.
140 The azimuth of the length axis of the transducer relative to the North of the

141 container was 37° and the elevation of the length axis of the transducer relative
142 to the base of the container was 17° , as shown in Figure 2.

143 *3.2 Ultrasound contrast agent*

144 DEFINITY® (Lantheus Medical Imaging, North Billerica, MA, USA) consists
145 of C_3F_8 gas microbubbles with mean diameters between 1.1 and $3.3\ \mu\text{m}$,
146 encapsulated by lipid/surfactant shells. Its resonance frequency had been
147 measured to be 2.7 MHz [19]. The 1.5-ml vials used in our experiments had
148 been stored at 9°C . Each vial was shaken for 45 s using a Vialmix® device
149 (Lantheus Medical Imaging). Before introducing the ultrasound contrast agent
150 in our setup, it was further diluted using a 0.9% saline solution.

151 The diluted ultrasound contrast agent was inserted using a syringe into
152 a microbore tube with a 0.51-mm inner diameter. The tube led to a
153 CUPROPHAN® RC55 cellulose capillary (Membrana GmbH, Wuppertal,
154 Germany) with a $200\text{-}\mu\text{m}$ inner diameter and an $8\text{-}\mu\text{m}$ wall thickness. The
155 middle of the capillary coincided with the optical focus of the objective lens
156 and with the acoustic focus of the ultrasound transducer, as shown in Figure 2.
157 The typical field of view using the $10\times$ objective lens was $500 \times 200\ (\mu\text{m})^2$,
158 whereas the diameter of the acoustic focus was greater than 5 mm. Hence,
159 the whole field of view could be considered in acoustic focus. The capillary
160 was positioned 2 mm from the base of the container. The flow speed of the
161 ultrasound contrast agent through the capillary was manually controlled.

162 In total, 48 experiments were performed. Bubble and cluster sizes were
163 measured and tracked using Image-Pro Plus (Media Cybernetics, Inc.,
164 Bethesda, MD, USA). Further analysis was done using MATLAB® (The
165 MathWorks, Inc., Natick, MA, USA).

166 4 Results and Discussion

167 At the high concentrations we used, clustering started instantaneously after
168 the ultrasound generator was switched on. Figure 3 illustrates the speed of
169 cluster formation of DEFINITY® ultrasound contrast agent that had been
170 further diluted to 1:20. With distances between the microbubbles of only few
171 micrometres, collision times from (11) should be within a second, indeed, as
172 shown in Figure 4. Also, from (11) and Figure 4 it is explained why cluster
173 formation must be faster at higher frequencies, if the other acoustic parameters
174 and the concentration are not changed. Or, after a fixed duration, larger
175 clusters must have formed using higher frequencies, since bubble can approach
176 from larger d_0 at higher f . These deductions are confirmed by our experimental
177 observations: In Figure 3, after 233 ms two clusters had been formed of
178 approximately $15\ \mu\text{m}$ each. These started to approach in the subsequent
179 frames. Overall, newly-formed clusters collided to form larger clusters. This is
180 illustrated by Figures 5 and 6. Each branch represents a cluster. The branches
181 coming together represent the collision of clusters into larger clusters. The
182 velocities of the clusters are on the order of tens of micrometres per second.
183 Although increasing the acoustic pressure would increase the cluster velocities
184 dramatically, as is evident from (13), they would also lead to microbubble
185 disruption [14]. We did not observe phenomena associated with microbubble
186 disruption.

187 The larger a cluster grows, the lower its resonance frequency becomes.
188 Hence, the velocity of a cluster in the direction of the sound field, defined
189 by (13), should decrease in time. If two identical clusters with resonance
190 frequency f_0 merge, the resulting resonance frequency $f'_c \approx \left(2^{-\frac{1}{3}}\right) f_0 =$
191 $0.79 f_0$ [1]. Assuming that the compressibility and damping coefficient do
192 not substantially change, a similar decrease in cluster velocity is expected.
193 However, the decrease in slope magnitude of the main branch in Figure 5 is
194 negligible. This might be explained if the resulting cluster is much stiffer than
195 the original clusters, increasing the damping coefficient.

196 Also, 7 MHz must be further off the cluster resonance frequency than 2 MHz.
197 Hence, the magnitudes of the slopes in Figure 6 are lower than those than
198 in Figure 5. Secondary radiation forces of clusters onto each other do not
199 explain the cluster colliding times observed. Even if the compressibility of
200 the clusters would be equal to that of a single ultrasound contrast agent
201 microbubble, under the acoustic conditions we used, the collision times from
202 (11) would be just milliseconds. Hence, the bubble clusters cannot be regarded
203 as identical monopoles in our setting. A close-up of two colliding clusters with
204 $22\text{-}\mu\text{m}$ diameters forming a $25\text{-}\mu\text{m}$ cluster is shown in Figure 7. The total time
205 spanning this process is slightly less than 1.8 s.

206 The clusters were initially formed in the middle of the capillary. These clusters
207 were located at distances $d_0 < \frac{1}{4}\lambda$, as demonstrated in Figure 8. However,
208 following further cluster coalescence during 17.55 seconds of sonication, the
209 final distance between the larger clusters corresponded to $\frac{1}{4}\lambda = 54 \mu\text{m}$. These
210 had been pushed towards the lower capillary wall, owing to primary radiation
211 forces.

212 The cluster velocities towards the capillary wall were between $5 \mu\text{m s}^{-1}$ at
213 7 MHz and 22 kPa peak-negative pressure and $15 \mu\text{m s}^{-1}$ at 2 MHz and 20 kPa
214 peak-negative pressure sonication. These are of the same order as the left-hand
215 side term in (13). The magnitudes of the slopes in Figure 6 did not change
216 close to the capillary wall. Hence, in our experimental setup, we neglected any
217 effect of the capillary wall on cluster translation. With cluster diameters less
218 than $30 \mu\text{m}$, buoyancy effects may neglected on our timescales as well.

219 In summary, we observed the following stages of microfoam formation,
220 illustrated in Figure 9. Our initial situation was a dense, random bubble
221 distribution before ultrasound arrival. After the sonication started, contrast
222 microbubbles collided, owing to secondary radiation forces. Subsequently,
223 these clusters coalesced within the space of a quarter of the wavelength,
224 owing to primary radiation forces. The resulting microfoams translated in
225 the direction of the ultrasound field, owing to primary radiation forces.

226 Small deviations in microbubble sizes or shell properties lead to deviations
227 in individual bubbles's resonance frequencies, as expressed in (8). These in
228 turn cause oscillation phase differences, as expressed in (6), big enough to
229 be optically observed [7]. Therefore, predicting and manipulating individual
230 microbubbles is technically challenging. We have demonstrated that as soon
231 as the bubble clusters were formed and as long as they were in the sound
232 field, they behaved as one entity. At our acoustic settings, it took seconds to
233 force the bubble clusters to positions approximately $\frac{1}{4}\lambda$ apart. It also just took
234 seconds to drive the clusters towards a boundary.

235 We may assume that vessel blocking can only be successful if a microfoam is
236 created with a diameter equal to or greater than the vessel diameter d_v . From
237 this study it follows that in order to create such a foam, $\frac{1}{4}\lambda > d_v$, or, $f < \frac{c}{4d_v}$.

238 For therapeutic purposes, it would be of great interest to induce microjetting
239 on entire clusters towards a vessel wall, presumably causing sonoporation or
240 sonolysis. Although ultrasound-induced microjetting has been observed with
241 ultrasound contrast agents, its occurrence in *in vivo* situations is hard to
242 control [20,21]. Predictable sonic manipulation would be better feasible if the
243 microbubbles would be forced to clusters of known size and position first.

244 **5 Conclusions**

245 We observed the following stages of microfoam formation within a densely
246 populated concentration of microbubbles. After the sonication started,
247 contrast microbubbles collided, forming small clusters, owing to secondary
248 radiation forces. These clusters coalesced within the space of a quarter of
249 the ultrasonic wavelength, owing to primary radiation forces. The resulting
250 microfoams translated in the direction of the ultrasound field, hitting the
251 capillary wall, also owing to primary radiation forces.

252 We have demonstrated that as soon as the bubble clusters were formed and
253 as long as they were in the sound field, they behaved as one entity. At our
254 acoustic settings, it took seconds to force the bubble clusters to positions
255 approximately a quarter wavelength apart. It also just took seconds to drive
256 the clusters towards the capillary wall.

257 Subjecting ultrasound contrast agent microbubbles to a continuous
258 low-amplitude signal makes them cluster to known positions and known
259 microfoam sizes, allowing for straightforward sonic manipulation.

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317 **List of Figures**

318	1	Schematic overview of the experimental setup	17
319 320 321 322	2	Close-up of the sonication tank with coinciding sound, light beam, and objective focus (<i>top</i>) and definitions of the azimuth and elevation of the transducer relative to the North of the container (<i>bottom</i>).	18
323 324 325 326	3	Microfoam formation during continuous sonication at 2 MHz and 20 kPa peak-negative acoustic pressure. Each frame corresponds to a $120 \times 120 (\mu\text{m})^2$ area. Time $t = 0$ was defined by the start of the sonication.	19
327 328 329 330	4	Collision times of individual encapsulated microbubbles as a function of driving frequency at given distances d_0 , using $p_a = 20 \text{ kPa}$, $R_0 = 1.25 \mu\text{m}$, $\kappa = 5 \times 10^{-6} \text{ m}^2 \text{ N}^{-1}$, and $\rho = 998 \text{ kg m}^{-3}$.	20
331 332 333 334 335 336 337 338	5	Cluster positions as a function of time, during continuous sonication at 2 MHz and 20 kPa peak-negative acoustic pressure. Position in the capillary is defined from East ($0 \mu\text{m}$) to West ($500 \mu\text{m}$). Bold lines indicate merged clusters. The beginning (<i>left</i>) of a line indicates the formation of a cluster of diameter $> 6.8 \mu\text{m}$. The end (<i>right</i>) of a line indicates the disintegration or contraction of a cluster to a diameter $< 6.8 \mu\text{m}$.	21
339 340 341 342 343 344 345 346	6	Cluster positions as a function of time, during continuous sonication at 7 MHz and 22 kPa peak-negative acoustic pressure. Position in the capillary is defined from East ($0 \mu\text{m}$) to West ($500 \mu\text{m}$). Bold lines indicate merged clusters. The beginning (<i>left</i>) of a line indicates the formation of a cluster of diameter $> 6.8 \mu\text{m}$. The end (<i>right</i>) of a line indicates the disintegration or contraction of a cluster to a diameter $< 6.8 \mu\text{m}$.	22
347 348 349 350 351	7	Two clusters, with $22\text{-}\mu\text{m}$ diameters and an initial distance of $55 \mu\text{m}$, colliding and coalescing during continuous sonication at a 2-MHz driving frequency and a 20-kPa peak-negative pressure. The frame size corresponds to $81 \times 81 (\mu\text{m})^2$. Times were relative to the start of the sonication ($t=0$).	23

352	8	Clusters forming during sonication at 7 MHz and 22 kPa	
353		peak-negative pressure. The frame size corresponds to	
354		$560 \times 264 (\mu\text{m})^2$. Time $t = 0$ was defined by the start of the	
355		sonication.	24
356	9	Schematic representation of the four stages of microfoam	
357		formation in a capillary: (<i>left-right</i>) random bubble	
358		distribution before ultrasound arrival, bubbles colliding during	
359		sonication, cluster coalescing within the space of a quarter of	
360		the wavelength, microfoam translation.	25

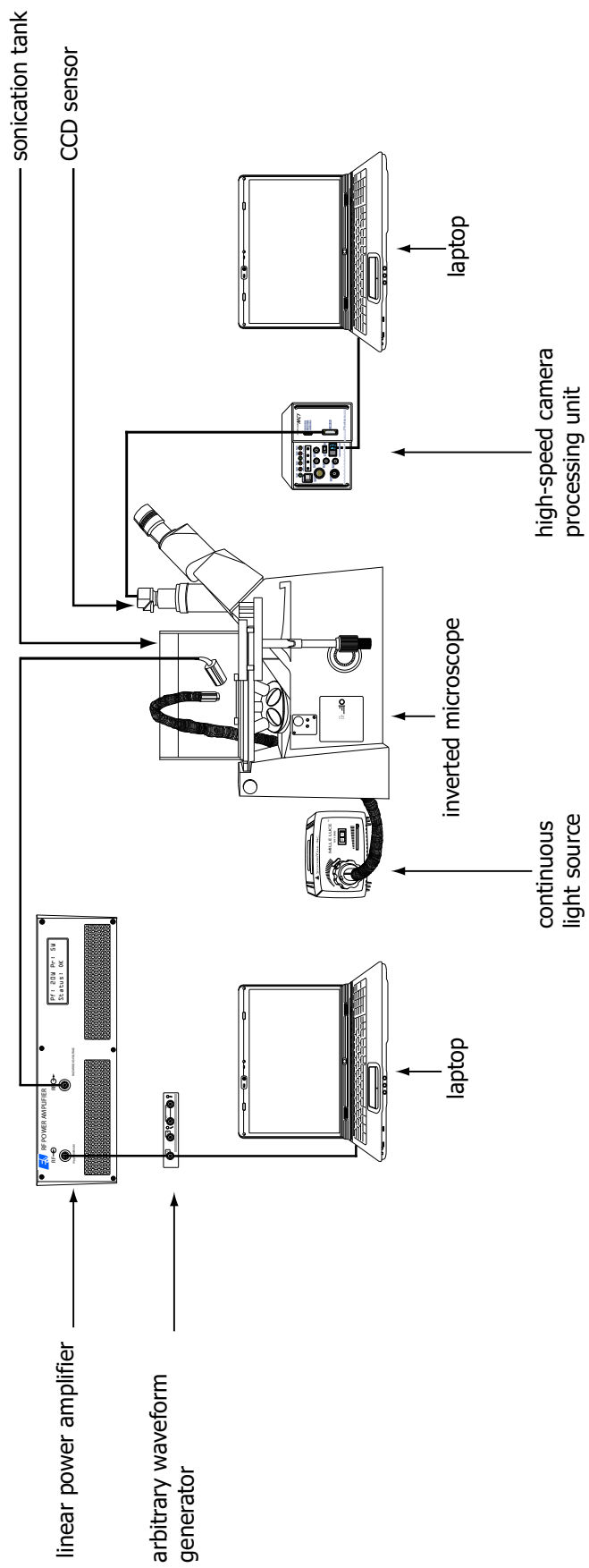


Fig. 1. Schematic overview of the experimental setup

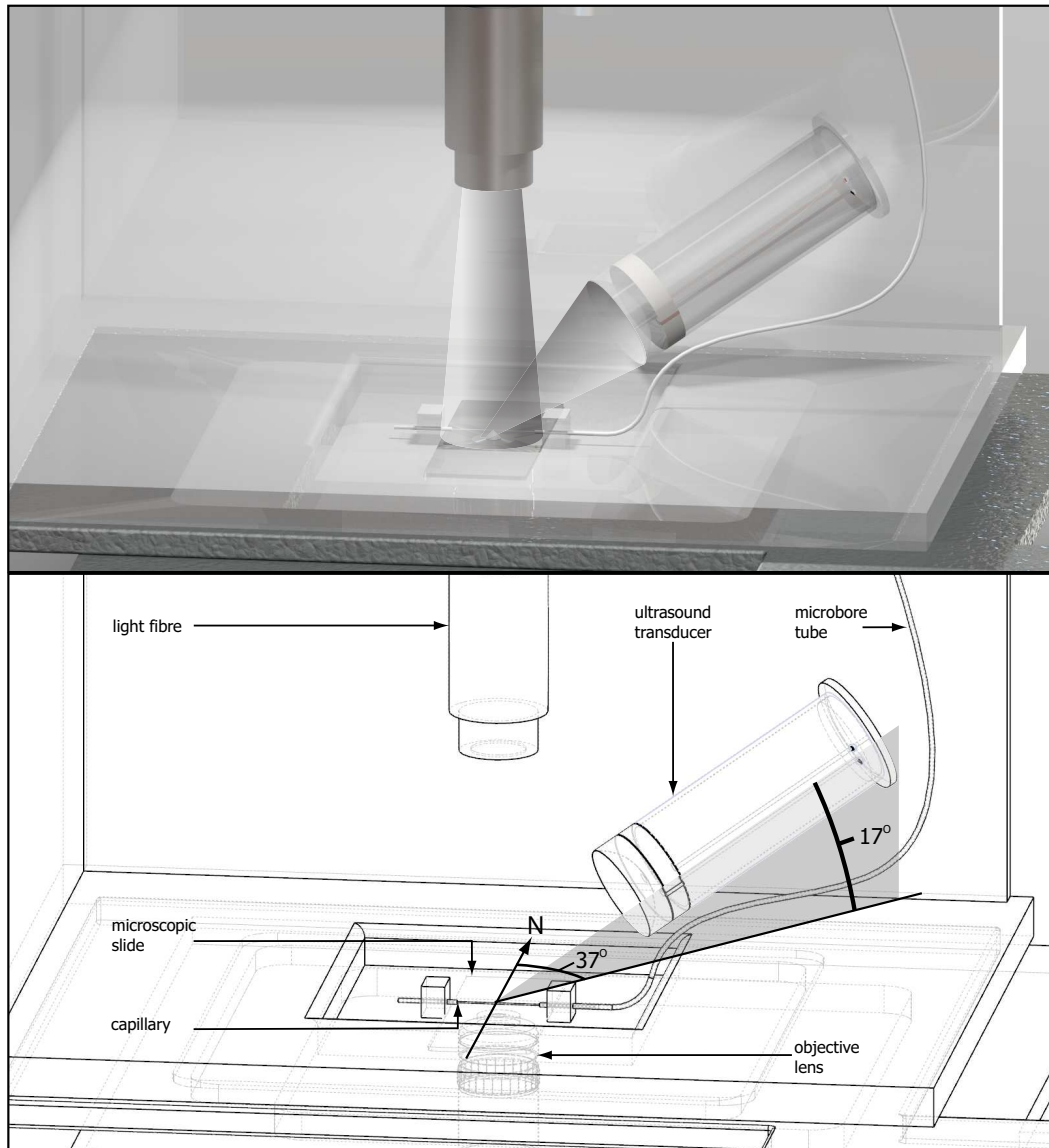


Fig. 2. Close-up of the sonication tank with coinciding sound, light beam, and objective focus (*top*) and definitions of the azimuth and elevation of the transducer relative to the North of the container (*bottom*).

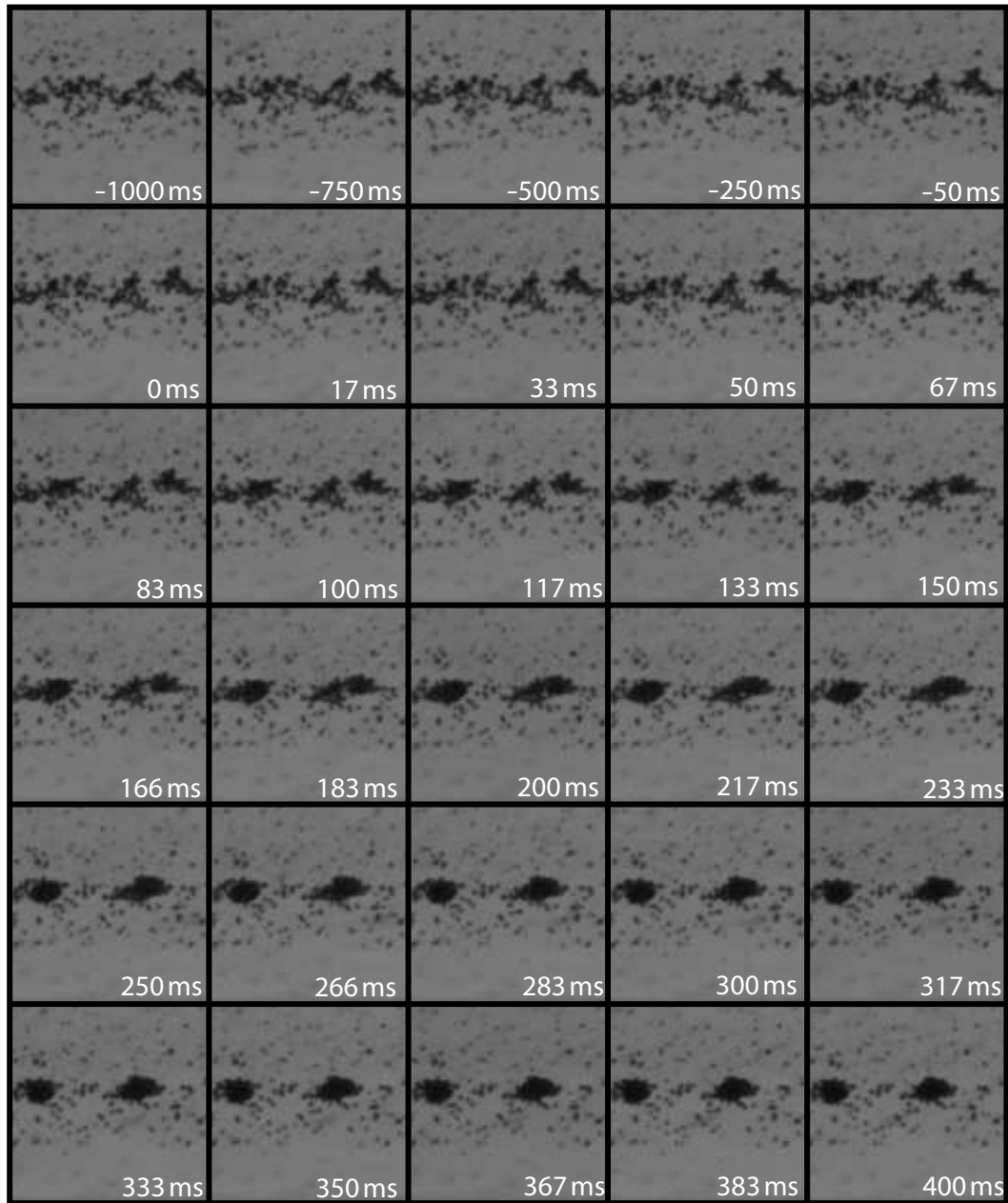


Fig. 3. Microfoam formation during continuous sonication at 2 MHz and 20 kPa peak-negative acoustic pressure. Each frame corresponds to a $120 \times 120 (\mu\text{m})^2$ area. Time $t = 0$ was defined by the start of the sonication.

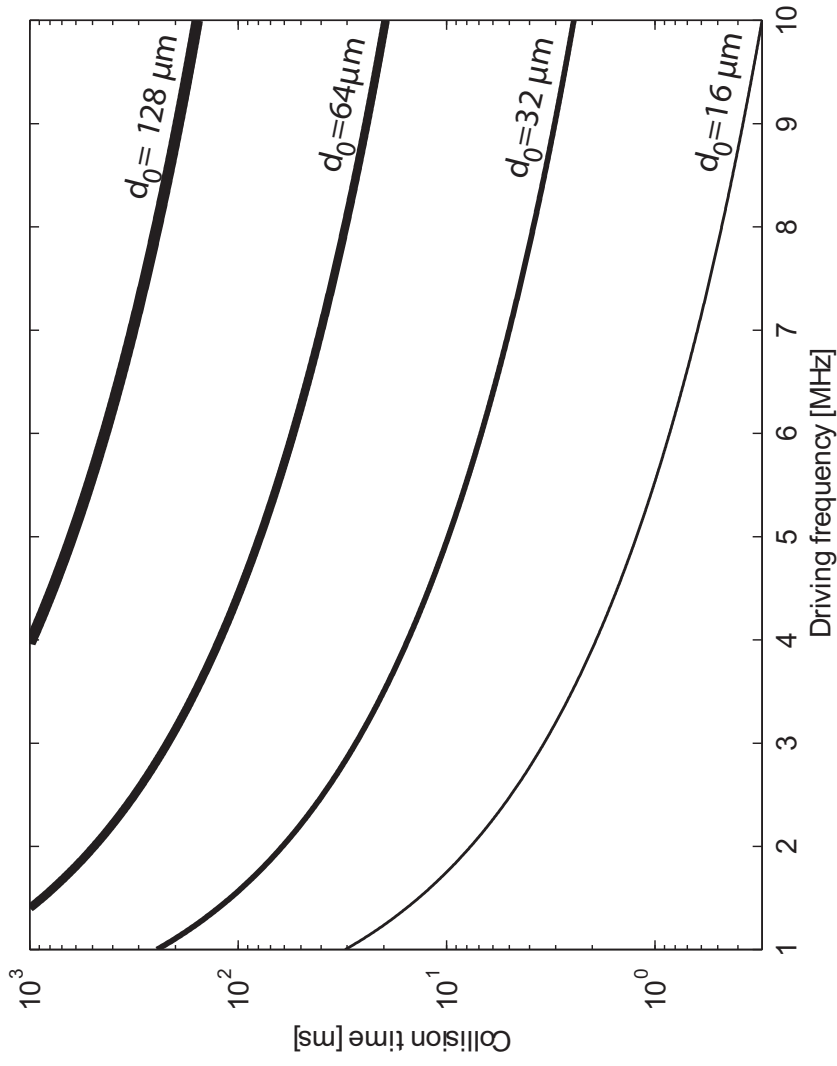


Fig. 4. Collision times of individual encapsulated microbubbles as a function of driving frequency at given distances d_0 , using $p_a = 20 \text{ kPa}$, $R_0 = 1.25 \mu\text{m}$, $\kappa = 5 \times 10^{-6} \text{ m}^2 \text{ N}^{-1}$, and $\rho = 998 \text{ kg m}^{-3}$.

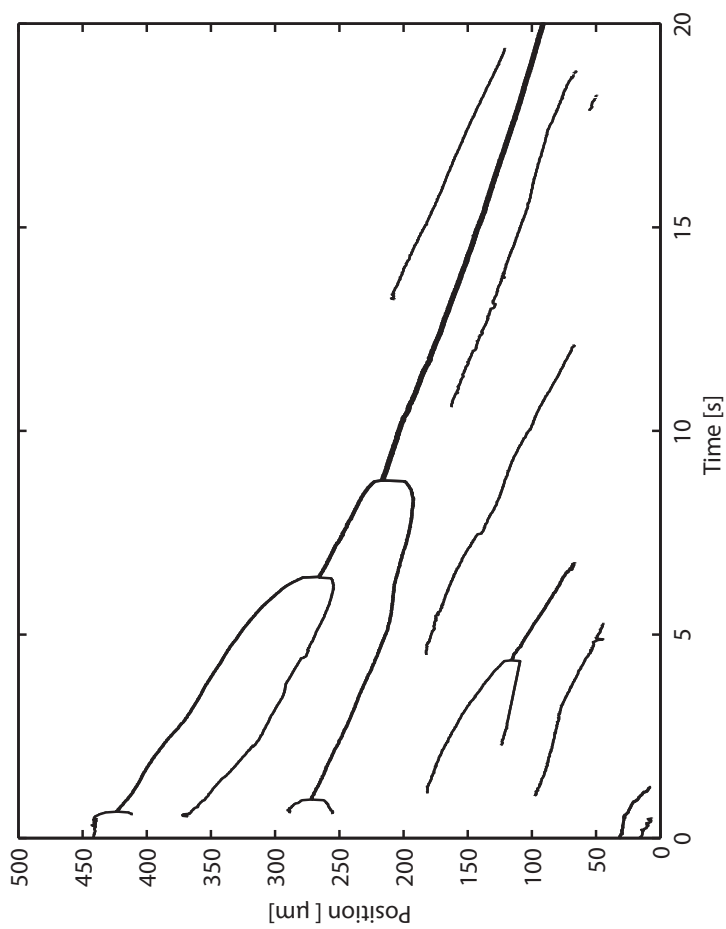


Fig. 5. Cluster positions as a function of time, during continuous sonication at 2 MHz and 20 kPa peak-negative acoustic pressure. Position in the capillary is defined from East ($0 \mu\text{m}$) to West ($500 \mu\text{m}$). Bold lines indicate merged clusters. The beginning (*left*) of a line indicates the formation of a cluster of diameter $> 6.8 \mu\text{m}$. The end (*right*) of a line indicates the disintegration or contraction of a cluster to a diameter $< 6.8 \mu\text{m}$.

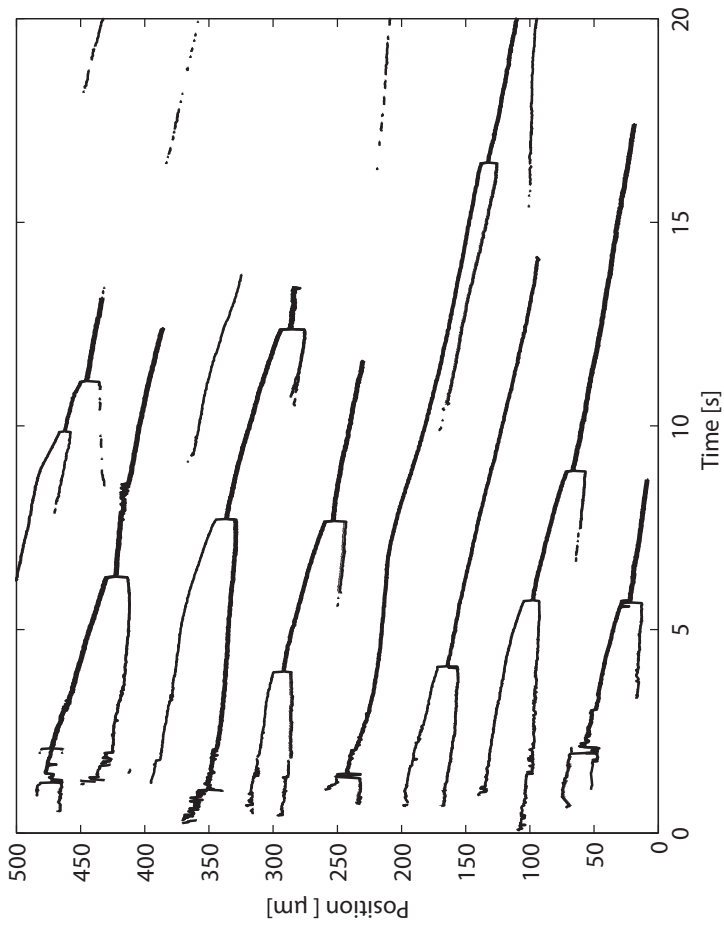


Fig. 6. Cluster positions as a function of time, during continuous sonication at 7 MHz and 22 kPa peak-negative acoustic pressure. Position in the capillary is defined from East ($0 \mu\text{m}$) to West ($500 \mu\text{m}$). Bold lines indicate merged clusters. The beginning (*left*) of a line indicates the formation of a cluster of diameter $> 6.8 \mu\text{m}$. The end (*right*) of a line indicates the disintegration or contraction of a cluster to a diameter $< 6.8 \mu\text{m}$.

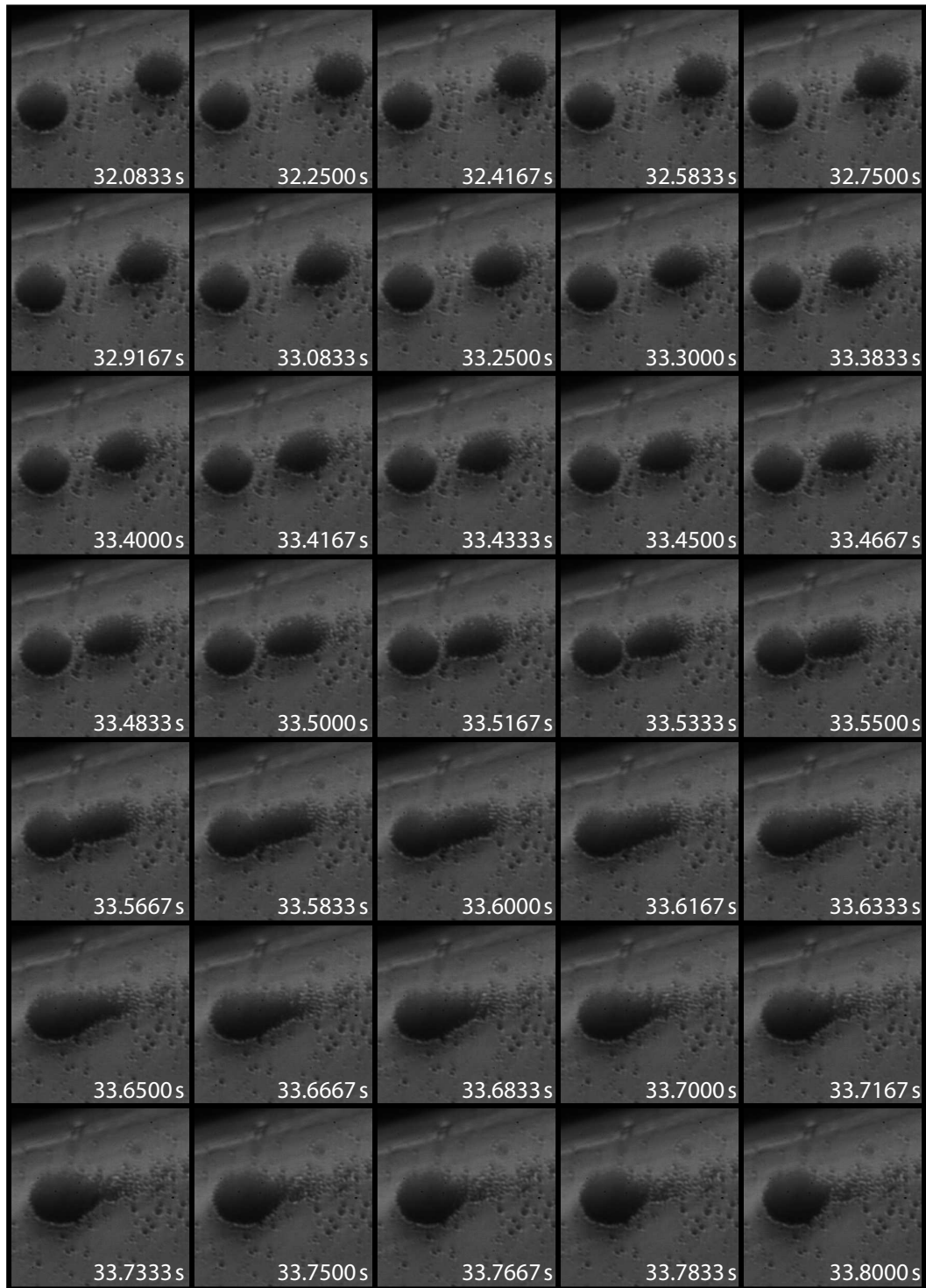


Fig. 7. Two clusters, with $22\text{-}\mu\text{m}$ diameters and an initial distance of $55\text{ }\mu\text{m}$, colliding and coalescing during continuous sonication at a 2-MHz driving frequency and a 20-kPa peak-negative pressure. The frame size corresponds to $81 \times 81\text{ }(\mu\text{m})^2$. Times were relative to the start of the sonication ($t=0$).

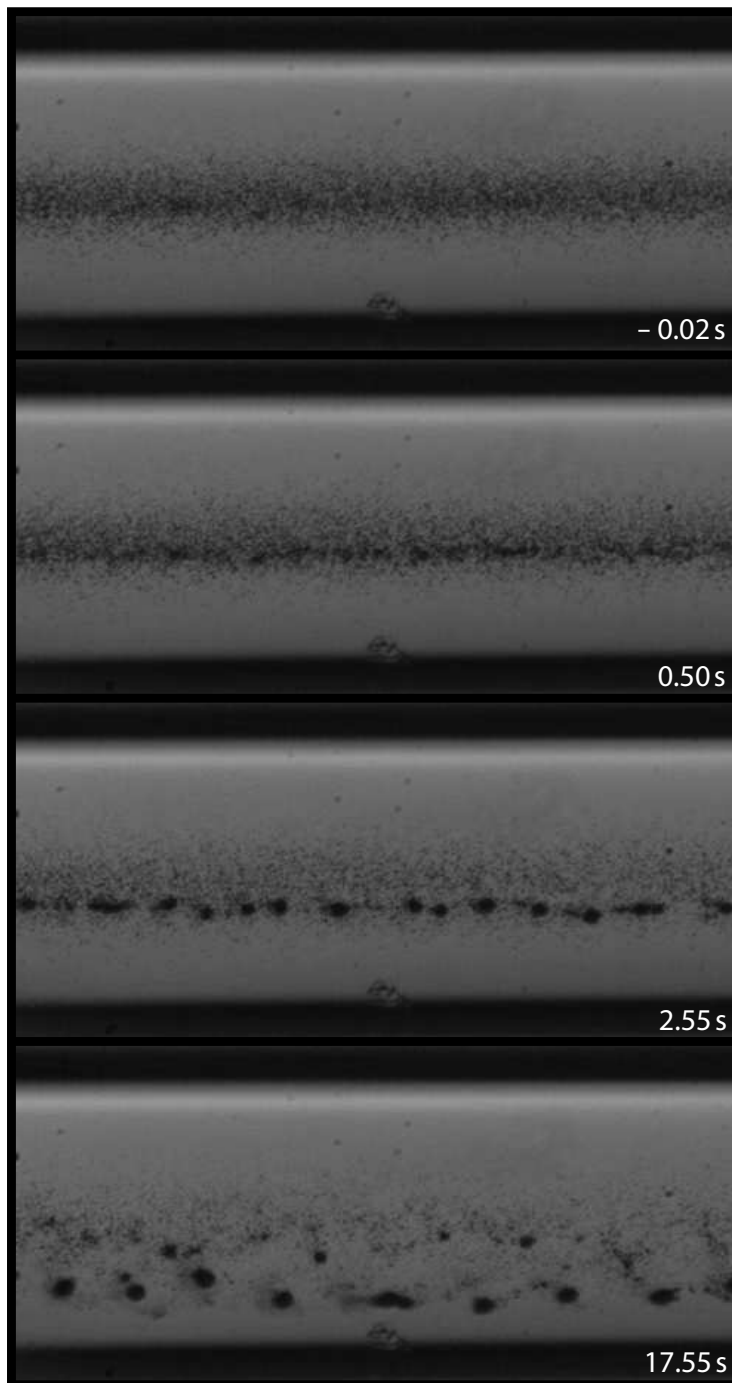


Fig. 8. Clusters forming during sonication at 7 MHz and 22 kPa peak-negative pressure. The frame size corresponds to $560 \times 264 (\mu\text{m})^2$. Time $t = 0$ was defined by the start of the sonication.



Fig. 9. Schematic representation of the four stages of microfoam formation in a capillary: (left-right) random bubble distribution before ultrasound arrival, bubbles colliding during sonication, cluster coalescing within the space of a quarter of the wavelength, microfoam translation.