Journal Name

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Cite this: DOI: 10.1039/c0xx00000x

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ARTICLE TYPE

Flow-focusing regimes for accelerated production of monodisperse drug-loadable microbubbles toward clinical-scale applications

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

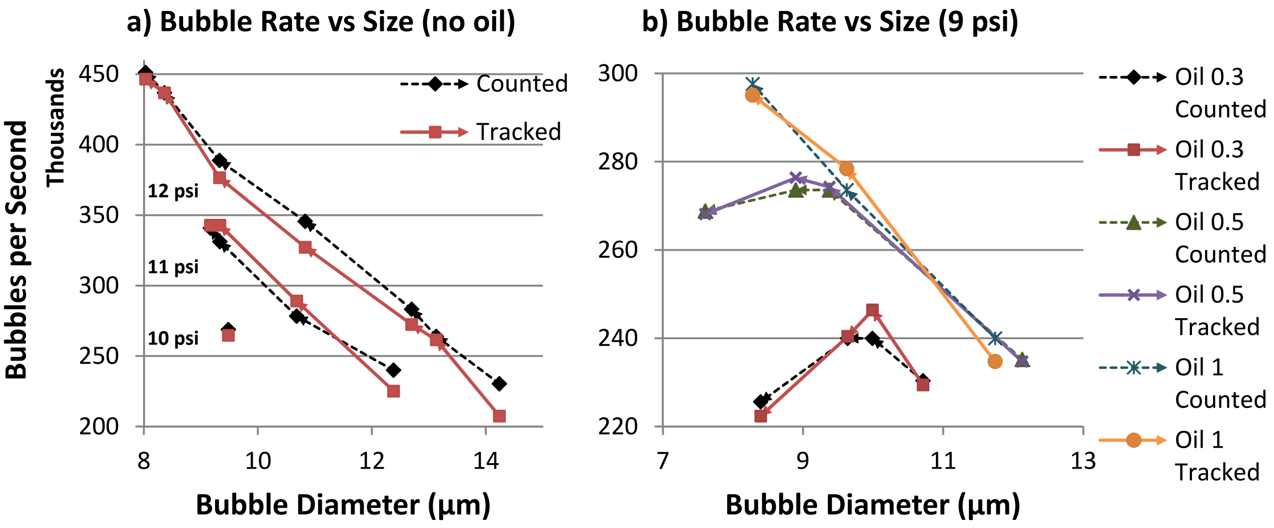


Fig. A1 Comparison of bubble production rates derived from the counting (dashed line) and tracking (solid line) methods. Counting data is same as Fig 5. a) No oil, at various pressures. b) With various oil flow rates, at 9 psi. The two measurement methods agreed well, differing by 6.2 kHz on average (2.6% of the camera's Nyquist frequency), with 6.3 kHz standard deviation (2.6%) and 22.9 kHz maximum (9.54%).

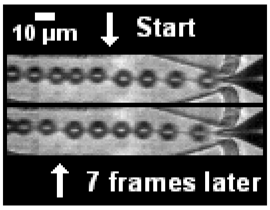
Appendix

Aliasing Mitigation

For production rates exceeding the camera's Nyquist frequency of 240 kHz (half the maximum recording frame rate), where aliasing can interfere with frequency measurements, results from counting were corroborated using the alternative method of:

$$bubs/sec = \frac{speed}{spacing} = \frac{displacement/frame * frames/sec}{bub\ center\ spacing}$$

where displacement was obtained by spotting and tracking distinctive features in the bubble stream:



Using this method, mean absolute error relative to counting results was 6.2 kHz, or about 2.6% of the Nyquist frequency, with a standard deviation of 6.3 kHz and a maximum error of 22.9 kHz (9.54% of the Nyquist frequency) (Fig A1). These results fall well within the margin of error needed to verify our measurements against aliasing, as explained below.

By aliasing theory, when a signal's frequency grows larger than the Nyquist frequency, an illusion called "folding" emerges where the frequency appears to decline in a mirror image of its pre-Nyquist growth, throwing off measurement interpretations. In our bubble production videos, however, this illusion manifests in a peculiar way that makes it easy to rule out:

1 Below Nyquist, apparent bubble speed increases along with production frequency. Since the stream of bubbles travels less than half a space forward between frames ($x < s/2$; see Fig A2), the human eye can track the motion without confusion.

2 At Nyquist, the direction of flow becomes hard to discern without context, since the bubbles travel exactly half a space forward ($x = s/2$), which appears identical to the bubbles traveling backward by the same distance.

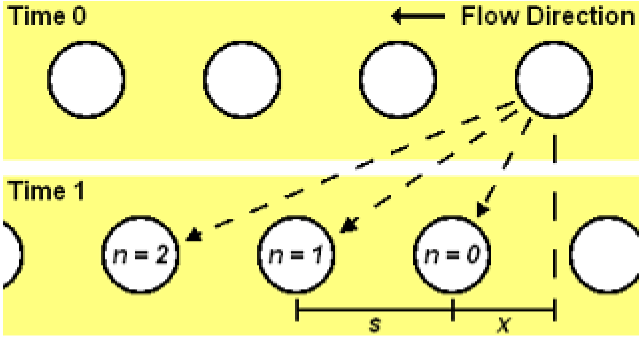


Fig. A2 Aliasing does not spell complete uncertainty. Given bubble spacing *s* and apparent displacement *x* between two video frames, the true displacement *D* must fulfill $D = ns + x$, where *n* is an integer ≥ 0, in order to align with the recording. This holds true even when production frequency exceeds half the framerate ($x > s/2$) and optical illusions of backward motion emerge. The possibility of backward motion can be ruled out since the flow is controlled by pump.

3. Above Nyquist, the bubbles travel over half a space forward ($x > s/2$), but the viewer may see an optical illusion of the bubbles traveling backward by a smaller distance ($s - x$). Furthermore, the "backward" flow appears to get slower as production frequency increases, since the illusory reverse-displacement ($s - x$) shrinks as forward-displacement *x* grows. This is the manifestation of symmetrical folding in our experimental setup, where frequency is bubble speed divided by center-spacing.

In abstract theory, there is no directionality to the signal being measured; only amplitude vs time. (Imagine a stationary pulsing light.) In our experiments, by contrast, we know the bubbles cannot move backward against the direction of lipid flow, since the latter is controlled by pump. This rules out the possibility of bubbles displacing backward by ($s - x$), and by extension, the possibility of frequency declining symmetrically above Nyquist.

Given that only forward motion is physically plausible, the domain of possible frequencies can be narrowed further, since the true frequency must align with the apparent displacement in the recorded frames. Specifically, when a stream of bubbles with spacing *s* (center-to-center) appears to displace forward by a certain distance *x* from one frame to the next, the true displacement *D* must satisfy:

$$D = ns + x$$

where *n* is an integer ≥ 0. (Fig A2)

Importantly, incrementing *n* implies a huge production-rate increase equivalent to the recording rate (480 kHz). To intuit this, consider the case $x = 0$, where ideally, one frame looks identical to the next. The possibilities are:

1. There is truly no movement ($n = 0$). This can be disproved with the naked eye.
2. The bubbles are moving forward exactly one place in line between each frame ($n = 1$), making room for one new bubble to emerge. This is a production rate of one bubble per frame, equal to the recording rate (480 kHz), or 2x Nyquist.
3. The bubbles are moving forward exactly n places in line between each frame ($n > 1$), making room for n new bubbles. This is a production rate of n bubbles per frame, or n * 480 kHz.

Thus, even an imprecise cross-checking method can identify the true *n*, as long as error is reasonably less than the Nyquist frequency, which it was in our case (Fig A1). *n* not exhibiting sudden jumps also makes physical sense, since lipid flow rate was always controlled via Harvard pump and raised in small increments.

Bubble Size Scaling

Scaling the bubble production data (Figs 3, 4) by gas fraction instead of gas-liquid ratio produces linear trends whose slopes vary with gas pressure (Figs A3, A4). The rescaled graphs resemble those of Cubaud and Ho (2004, Figs 16, 17), for flow-speed-dependent resistance and pressure-drop at liquid fractions >0.1. This suggests that variable resistance and pressure drop are involved in displacing the scaling curves, e.g. by altering bubble spacing.

In the rescaled graphs, both the slopes and the y-intercepts decrease with increasing pressure, with diminished incremental effects above ~15 psi. In other words:

1. Bubble diameter is directly proportional to gas fraction at each pressure.
2. At low pressures, an equivalent decrease in gas fraction comes from a greater decrease in bubble diameter than at high pressures. Bubble spacing also tightens more, proportionally to the cube of diameter, since:

$$\mathit{gas\ fraction} \propto \frac{\mathit{bubble\ volume}}{\mathit{bubble\ spacing}} \propto \frac{(\mathit{bubble\ diameter})^3}{\mathit{bubble\ spacing}}$$

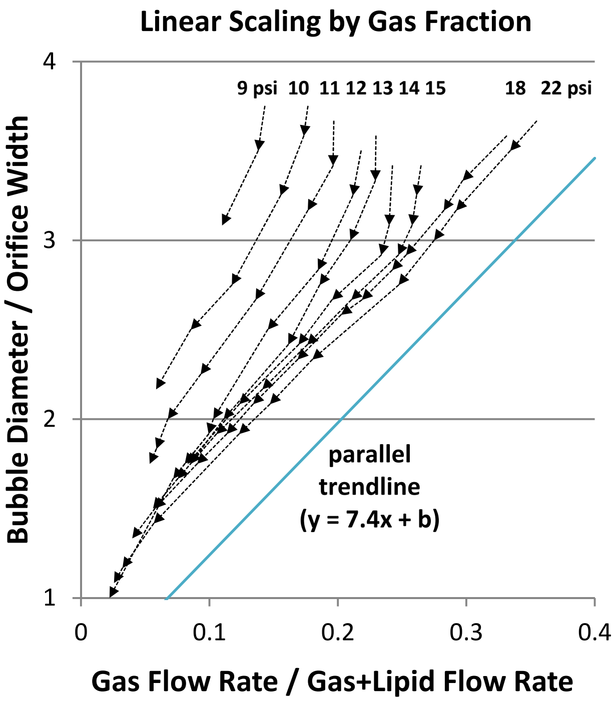


Fig. A3 Single-layer production scaled to gas fraction. At each pressure, scaling is roughly linear, with slope and y-intercept decreasing as pressure increases. Above 15 psi, slope changes less, remaining ~7.4.

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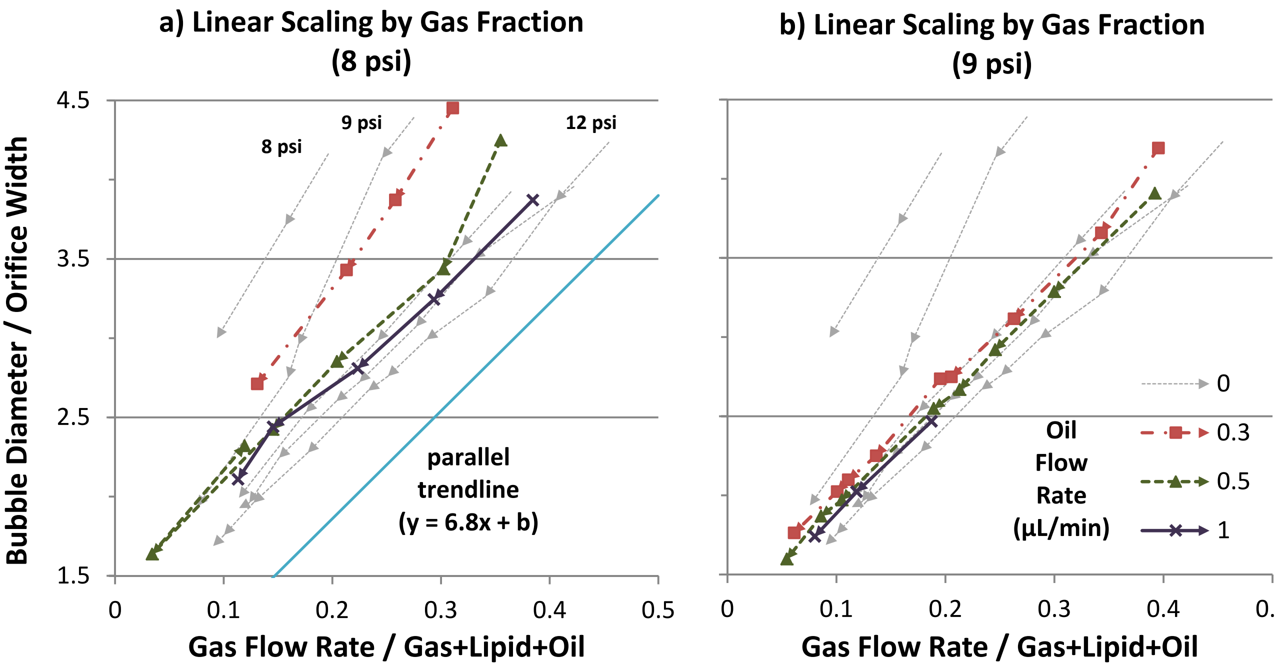

Fig. A4 Dual-layer production scaled to gas fraction. Trends are linear, with slope and y-intercept decreasing as oil is added, again similar to a 1-3 psi increase in gas pressure.

Notes

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