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Commentary

An arrayable flow-through microcentrifuge for high-throughput instrumentation–a matter of scale

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Often, technical advances are necessary for scientific progress. The recent paper by Marziali *et al.* (1) describes significant technical advances to one of the oldest biochemical methods, preparative ultracentrifugation. These advances are achieved by scaling the equipment and its operation to take advantage of the rigorous theory that underlies centrifugation.

To put this advance into context, it is important to realize that nearly every preparative protocol associated with subcellular fractionation involves preparative centrifugation at one or more steps. This widespread use of preparative centrifugation stems from its ability to separate larger and smaller components simply, rapidly, and gently. Protocols are easily developed because the velocity of a mass suspended in a viscous media subjected to a gravitational field depends on the ratio of a particle's mass to its frictional coefficient (2). Furthermore, because the scaling laws for centrifugation are rigorous, it is rather simple to adapt existing protocols to take advantage of the particular capabilities of any centrifuge. It is the certainty of these scaling laws that will allow the rapid adaptation of existing protocols for binding assays and for isolation of biological reagents to the arrayable microcentrifuge (1).

So what are these scaling laws, and how are they applied in this article? First, the gravitational field that drives sedimentation scales as $\omega^2 r$, where ω is the rotor angular velocity and r is the distance from the center of rotation. A consequence of this relationship is that only a twofold increase in rotor speed is needed to generate the same gravitational field in a rotor that is one-fourth the size. The authors have taken advantage of this scaling law to develop rotors only a few millimeters in diameter that are still capable of generating fields in excess of $20{,}000 \times g$. The notion of using smaller, faster rotors is not new (3). What has changed is the increased demand for methods to process large numbers of small-volume samples in parallel. The authors have answered this need by developing rotors small enough that they can be arranged to fit the dimensions of a 96-well plate, while still maintaining the capabilities of a full-fledged preparative centrifuge. These advances bring the power of centrifugation into low-volume, sample-intensive endeavors such as genome sequencing and combinatorial chemistry. What is perhaps most important is that an entire preparative protocol consisting of pelleting, resuspension, washing, and re-pelleting can be conducted automatically and with the retrieval of both the pellet and supernatant at each step.

Time also is of concern in the sample-intensive worlds of genome sequencing and combinatorial chemistry. Fortunately, shrinking the rotor size also reduces the time needed to complete a protocol. In particular, the time needed to sediment a certain distance scales as the inverse square of the rotor speed and as the log of distance. With rotor speeds of 70,000 rpm and distances of only a few millimeters, pelleting can be completed in a matter of seconds.

The small size of the rotor also allows the control of the hydrodynamic shear experienced during sedimentation. Low shear is desirable during sedimentation to ensure uniform, tight packing of the pellet. High shear is needed, though, to resuspend a pellet. The rate of shear is controlled by the rate of rotor acceleration. At high acceleration rates, the solution cannot keep up with the rotor, resulting in a turbulent flow in which the solution tumbles over the face of the pellet, and leading to a rapid and complete resuspension of the pellet. Because of the small inertia of the tiny rotors, it is possible to quickly reverse the direction of rotation—much like the spindle in a washing machine—thus creating enormous shear at the liquid–pellet interface. Perhaps it is this final bit of scaling that makes the development of this microcentrifuge so significant because it automates pellet resuspension—a traditionally manual task.

Taken together, the apparatus presented by Marziali *et al.* (1) allows preparative centrifugation to become a mainstream method in the massively parallel protocols that characterize so much of molecular biology today.

1. Marziali, A., Willis, T. D. & Davis, R. W. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 61–66.
2. Fujita, H. (1962) *Mathematical Theory of Sedimentation Analysis* (Academic, New York).
3. Svedberg, T. & Pedersen, K. O. (1940) *The Ultracentrifuge* (Johnson Reprint, New York).

The companion to this Commentary begins on page 61 in issue 1 of volume 96.

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