CENTRIFUGER: NON-TRIVIAL BALANCE OF CENTRIFUGE ROTORS

A PREPRINT

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Abstract

Centrifuges are indispensable instruments in biology laboratories. For the safe operation of centrifuges, ensuring balance is a must. Loading test tubes in opposite holes and adding dummy tubes are far from the only ways to balance the centrifuge rotors. The balance can be sought through higher-order symmetrical configurations of tubes, which offer operational advantages, particularly in dealing with tubes of unequal mass. Because these configurations are somewhat complicated, an open-source tool was introduced to assist centrifuge users in the adoption of this non-trivial approach.

***K*eywords** centrifuge problem · blank tube · prime number · linear combination · rotational symmetry · random sampling

1 Introduction

Centrifuges are essential devices for particle fractionation in biochemistry and molecular biology laboratories. Due to the extreme forces generated during operation, the use of centrifuges requires safety practices to eliminate potential associated hazards (Clark 2001). Accordingly, the importance of balancing test tubes in centrifuge rotors has been emphasized in classical texts on centrifugation (Birnie and Rickwood 1978; Rickwood 1989; Ford and Graham 1991; Graham 2001) as well as in laboratory manuals (World Health Organization 2003). Specifically, a rotor is balanced when the center of mass of the test tubes and the center of rotation of the rotor coincide (Johnsson 2016; Baker 2018; Blinder 2020). For popular fixed-angle rotors with even numbers of equally spaced holes such as 24 and 30, an even number of identical test tubes are conventionally balanced by loading tubes in opposite holes (Fig. 1a). To balance an odd number of test tubes, the common practice (Birnie and Rickwood 1978; World Health Organization 2003) is to add a "dummy" tube containing water or the same solution as the test tube(s) as a counterweight (Fig. 2a).

The search for alternative methods to balance the odd numbers of test tubes without using the dummy tube gave rise to the "balanced centrifuge problem" (Baker 2018, 2022; Krieger 2018; Runia 2020) which originally questioned which k identical test tubes can be balanced in an n-hole centrifuge rotor. Sivek (2010) proved that the balance is perfectly possible "if and only if both k and $n - k$ are expressible as linear combinations of prime factors of n with nonnegative coefficients". For example, 7 identical test tubes can be balanced in a 30-hole rotor if and only if 7 and 23 can be expressed as a sum of 2, 3, and 5. The involvement of these prime numbers stems from the fact that the configurations of tubes with rotational symmetry of orders 2, 3, and 5 are intrinsically balanced in a 30-hole rotor since their mass centers always coincide with the rotor rotation center (Fig. 1). Consequently, the superimposition of these configurations also maintains the balance (Fig. 2b, c) as long as there is no overlap of test tubes in the same hole, i.e., the exclusion principle (Peil and Hauryliuk 2010; Baker 2018).

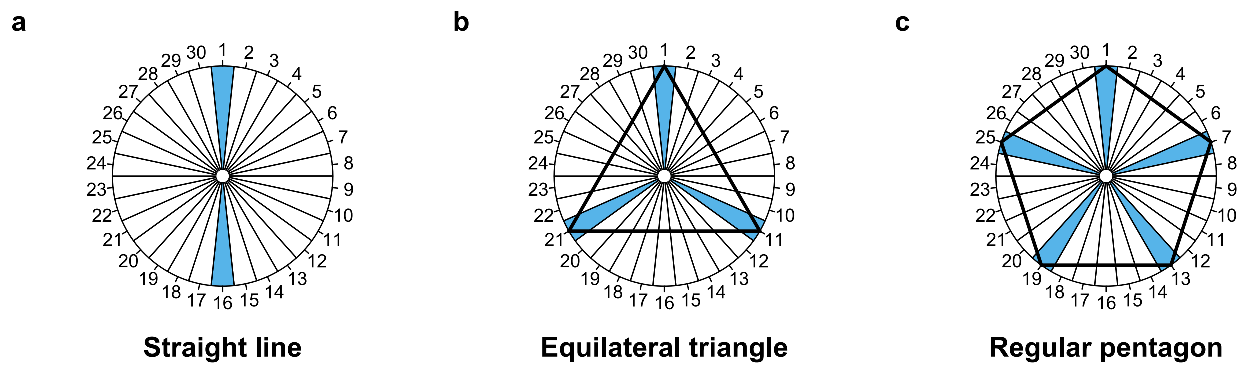

Figure 1: Balanced configurations of identical test tubes with rotational symmetry of orders 2, 3, and 5 in a 30-hole rotor, forming a straight line, an equilateral triangle, and a regular pentagon, respectively. Filled holes are marked with colors.

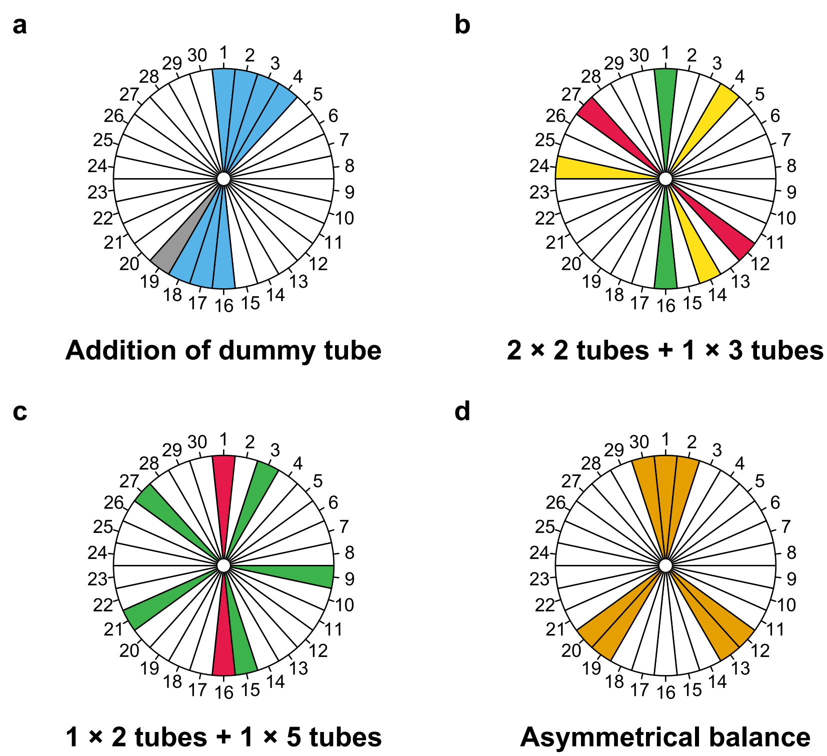

Figure 2: Three approaches to balance 7 identical test tubes in a 30-hole rotor – adding a dummy tube (hole 19) to create balanced configurations with rotational symmetry of order 2 (a), using balanced configurations with rotational symmetry of higher orders (b, c), and using asymmetrical configurations (d). Filled holes are marked with colors. Holes that form a configuration with rotational symmetry (b, c) share the same color.

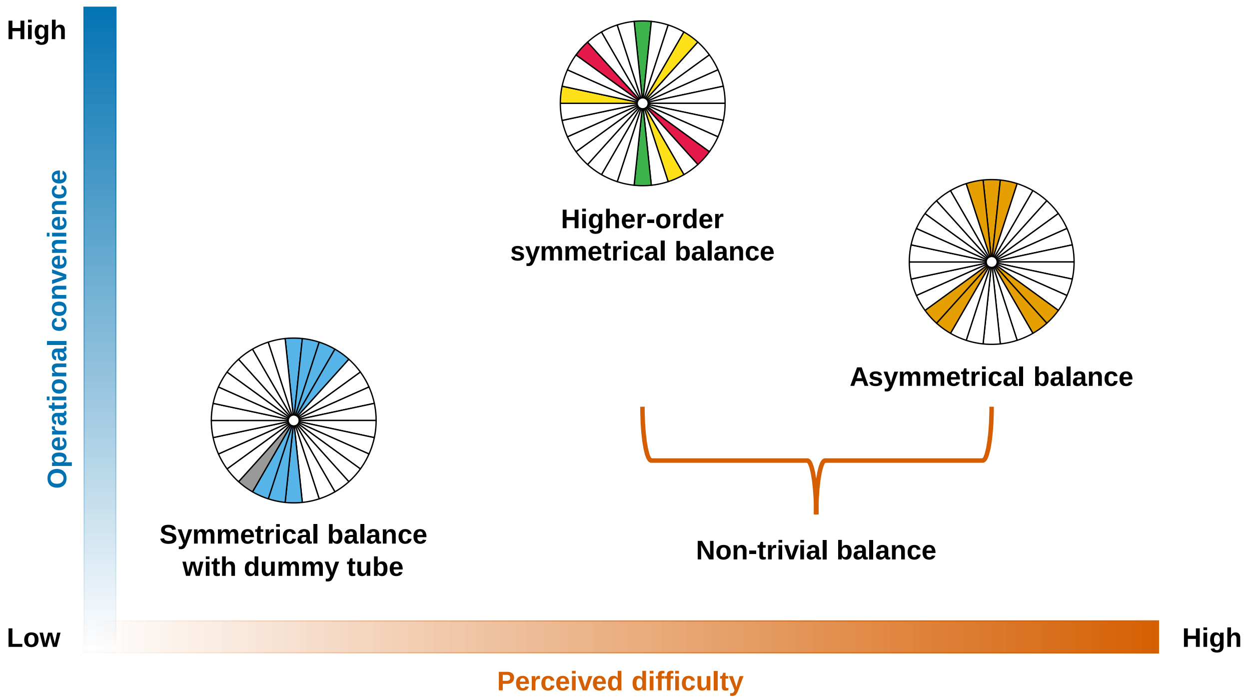

Figure 3: A comparison of three approaches to balance the odd numbers of identical test tubes in centrifuge rotors in terms of perceived difficulty and operational convenience.

Based on the theorem of Sivek (2010), several efforts have been made to find the rotationally symmetrical balance of k tubes in centrifuge rotors, providing one or multiple solution(s) for each valid k (Lundberg 2019; O'Neill 2020; Hernández 2021; Gao 2022). In fact, the 30-hole rotor supports the symmetrical balance of all possible numbers of tubes except 1 and 29 (Lundberg 2019). Notably, Peil and Hauryliuk (2010) showed that the balance can also be achieved with many asymmetrical configurations of tubes that are seemingly not built upon the tube configurations with rotational symmetry (Fig. 2d). However, the asymmetrical balance of 7 tubes, for example, is merely one possible result of removing 23 tubes with rotationally symmetrical configurations from a fully filled 30-hole rotor. The next section discusses the merits of symmetrical configurations (with or without the dummy tube) and asymmetrical configurations in balancing centrifuge rotors.

2 The Trivial, the Promising, and the Unnecessary

Adding a dummy tube to form the trivial configurations with rotational symmetry of order 2 (Fig. 2a) is the most intuitive approach to balancing the odd numbers of test tubes (Fig. 3). Without the dummy tube, the symmetrical balance of tubes must entail the configurations with rotational symmetry of higher orders (Fig. 2b, c), making it more difficult to grasp. However, what perplexes users the most is the asymmetrical configurations (Fig. 2d), which would require more than mental calculation to verify the balance (Peil and Hauryliuk 2010). Given the high perceived difficulty (Fig. 3), the non-trivial configurations (i.e., asymmetrical and higher-order symmetrical) should only be utilized by well-informed users.

Operationally, preparing a dummy tube of the same mass as a test tube could be time-consuming. The non-trivial configurations obviate the need for the dummy tube and thus are more convenient (Fig. 3). However, the asymmetrical configurations are practically unnecessary since they offer no operational advantage while being overly complicated compared with the higher-order symmetrical configurations. Seeking higher-order symmetrical balance is a promising practice, as explained in the next section.

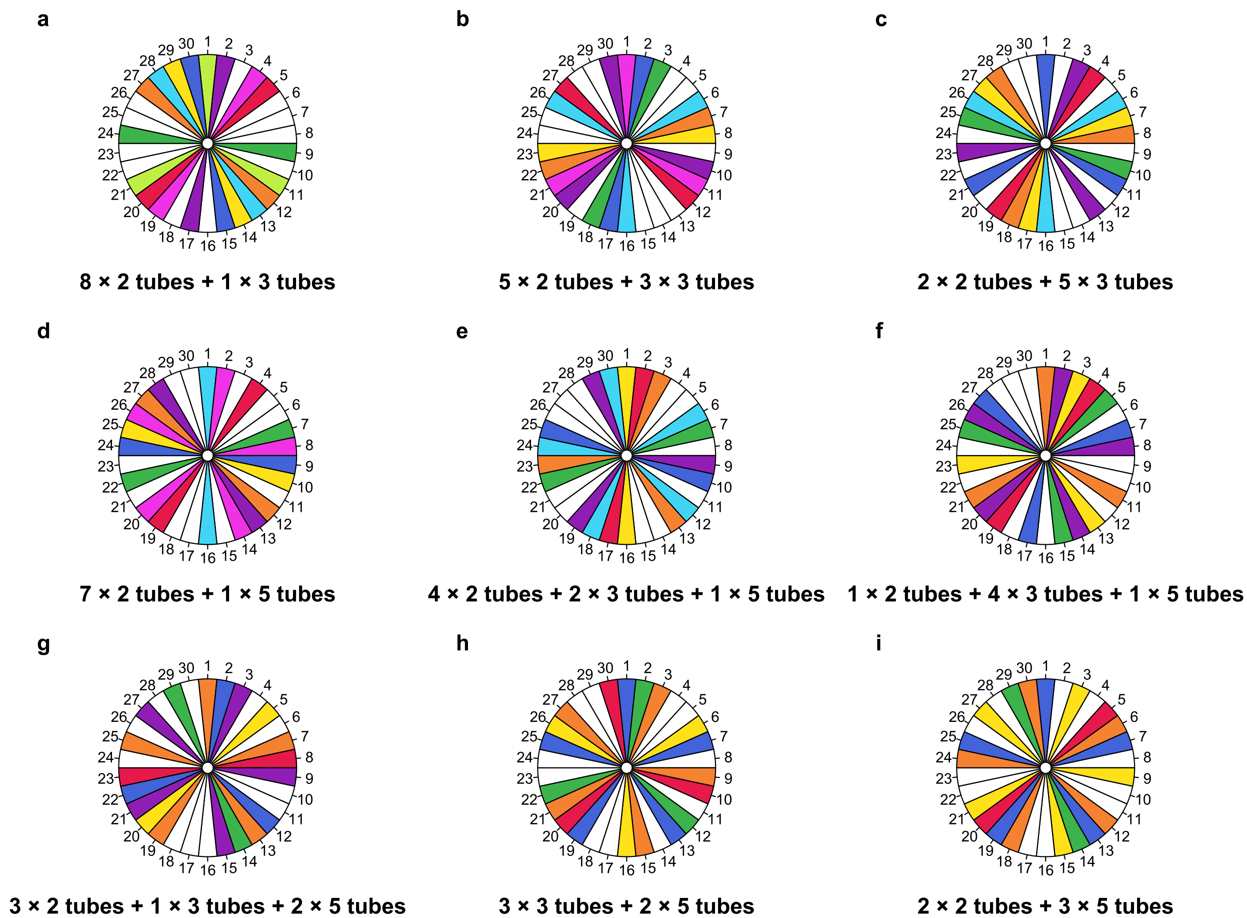

Figure 4: Nine ways to balance 19 test tubes in a 30-hole rotor by the decomposition into the sets of 2, 3, or 5 tubes, which are advantageous in handling non-identical tubes. Filled holes are marked with colors. Holes that form a configuration with rotational symmetry share the same color.

3 Inequality

One possible application of the higher-order symmetrical configurations is the balancing of non-identical test tubes (i.e., tubes of unequal mass). For instance, in the case of 7 tubes in a 30-hole rotor (Fig. 2b), doubling the mass of the three tubes belonging to the equilateral triangle configuration does not alter the center of mass of all 7 tubes. The balance can be sustained as long as the tubes that form a configuration with rotational symmetry have the same mass (Fig. 1). It is worth noting that there is more than one way to split 7 tubes into sets of 2, 3, or 5 tubes (Fig. 2b, c), giving more flexibility in handling tubes of unequal mass.

Previous works on the rotationally symmetrical balance without the dummy tube (Lundberg 2019; O'Neill 2020; Hernández 2021; Gao 2022), unfortunately, have only focused on determining which rotor holes to be filled to achieve the balance but overlooked the diversity of symmetrical configurations. The R package *centrifugeR* was introduced in 2020 to address this issue. Briefly, *centrifugeR* used simple random sampling to list different ways to express k test tubes as a sum of prime factors of n holes. For example, 19 test tubes in a 30-hole rotor can be decomposed in 9 unique ways (Fig. 4). The mechanics of *centrifugeR* are demonstrated with $n = 30$ and $k = 19$ and summarized in the next section. As k increased, more ways of decomposition into prime factors were often expected (Fig. 5). Some of those ways, however, were invalid due to the exclusion principle (Peil and Hauryliuk 2010; Baker 2018). For example, 23 can be theoretically expressed as a sum of 2, 3, and 5 in 13 different ways but only 8 of those actually worked in the 30-hole rotor (Fig. 5).

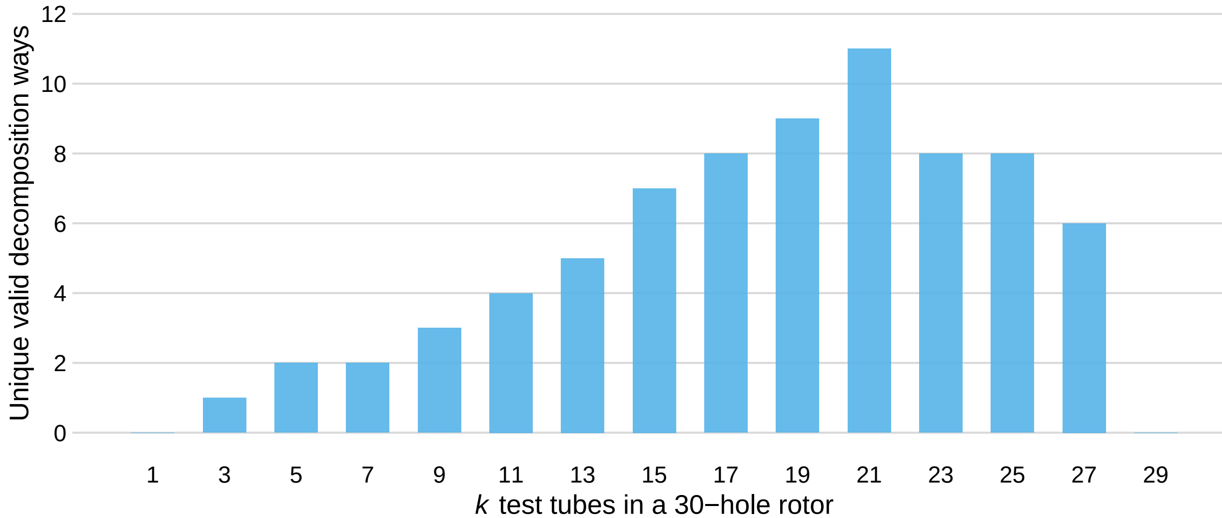

Figure 5: The number of unique valid ways to decompose the odd numbers of test tubes into the sets of 2, 3, or 5 tubes in a 30-hole rotor.

4 Mechanics of *centrifugeR*

Step 1. Find the prime factors p of $n = 30$: $p = \{2, 3, 5\}$.

Step 2. Find the possible coefficients a of each p with $\max(a) = \frac{n}{p}$:

$$a = \begin{cases} \{a_1 \in \mathbb{N} \mid 0 \leq a_1 \leq 15\} & \text{if } p = 2 \\ \{a_2 \in \mathbb{N} \mid 0 \leq a_2 \leq 10\} & \text{if } p = 3 \\ \{a_3 \in \mathbb{N} \mid 0 \leq a_3 \leq 6\} & \text{if } p = 5. \end{cases}$$

Step 3. Compute the values LC of the linear combination of $p_1 = 2$, $p_2 = 3$, and $p_3 = 5$ with coefficients a:

$$LC = a_1p_1 + a_2p_2 + a_3p_3.$$

Step 4. Check if both $k = 19$ and $n - k = 11$ appear in the list of $(15 + 1)(10 + 1)(6 + 1) = 1232$ values of LC. If yes, return the sets of coefficients a_1, a_2, and a_3 corresponding to the locations of $k = 19$ in the list (Table 1).

Table 1: Coefficients a in 9 ways to express 19 as a sum of 2, 3, and 5.

Location	$p_1 = 2$	$p_2 = 3$	$p_3 = 5$
25	8	1	0
54	5	3	0
83	2	5	0
184	7	0	1
213	4	2	1
242	1	4	1
372	3	1	2
401	0	3	2
531	2	0	3

Step 5. Find the sets of rotor holes that form straight lines, equilateral triangles, and regular pentagons (Fig. 1) corresponding to $p_1 = 2$, $p_2 = 3$, and $p_3 = 5$, respectively:

$$\begin{cases} \{1, 16\}, \cdots, \{15, 30\} & \text{if } p = 2 \\ \{1, 11, 21\}, \cdots, \{10, 20, 30\} & \text{if } p = 3 \\ \{1, 7, 13, 19, 25\}, \cdots, \{6, 12, 18, 24, 30\} & \text{if } p = 5. \end{cases}$$

Step 6. For each location of $k = 19$, randomly sample without replacement a_1 out of 15 sets of straight line-forming holes, a_2 out of 10 sets of equilateral triangle-forming holes, and a_3 out of 6 sets of regular pentagon-forming holes, e.g., $a_1 = 8$, $a_2 = 1$, and $a_3 = 0$ in the case of location 25 (Table 1). Repeat the random sampling process until all 19 obtained holes are different (i.e., no duplicates of holes) or until a time limit is reached.

Step 7. Visualize $k = 19$ tubes as the sets of 2, 3, or 5 tubes in centrifuge rotors (Fig. 4).

5 Accessibility

centrifugeR is written in R (R Core Team 2022) and publicly available on CRAN at `https://cran.r-project.org/package=centrifugeR` and on GitHub at `https://github.com/phamdn/centrifugeR`. A web application is available at `https://phamdn.shinyapps.io/centrifugeR/`.

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