Supplementary Note 2.

The coding capacity (*C*) is determined by the color number (*L*) and the labelled branch number (*P*), and calculated by the following formula: $C = (P+L-1)! / [P!(L-1)!]$, where *P* is determined by the generation number (*n*) of DL-DNA[14] ($P = 3x2^n$). In theory, 325 different nanobarcodes can be fabricated with three fluorescent colors and with a third generation (G_3) DL-DNA as fluoro-dye carrier. However, in practice, the number of codes that can be distinguished may be much fewer due to factors such as fluorescence resonance energy transfer (FRET), choices of dyes, equipment sensitivity and signal to noise ratios, and detection methods. For example, due to FRET, two dyes may not be conjugated onto the same end of a dsDNA branch. In addition, the detection sensitivity of certain equipment may not be high enough to resolve two codes that are nearly similar, such as 5Green4Red vs. 16Green12Red whose respective ratios (Red/Green) are 1.25 and 1.33. Furthermore, detection methods can also affect the coding capacity. For example, two codes 4Green1Red and 8Green2Red are not distinguishable on a population-level as demonstrated in this paper, yet they can be differentiated using single molecular detection (data not shown). In our flow data (**Fig. 4**), we were able to distinguish clearly those nanobarcodes whose ratio of fluorescence was more than 4-fold different. The coding capacity increased with the fluorescence microscopy (see **Supplementary Fig. 3** online), where we were able to distinguish those nanobarcodes whose ratio of fluorescence was more than 2-fold different. Multiplex detection is also influenced by hybridization conditions. Taken together, with optimized conditions, the possibility of fabricating higher generation DL-DNA and using a 4^{th} color, the multiplexing capacity of DL-DNA should be adequate for most applications.