Andrew Leifer PhD Thesis
Supplementary Information

Chapter:
Self-Assembled Geometrically Encoded DNA Fluorescence Nano-barcodes

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Supplementary Tables

Table S.1.1 Handle sequences for rod-like barcodes
Staple extension sequences used for constructing the 800-nm nano-barcode and 400 nm super-resolution barcode. The staple extension names are colored to match the staples in the strand diagram shown in Figure S1. Handle 1 and 2 are located at the front monomer and handles 4–6 are at the rear monomer (Figure S1a). The 3'-ends of biotinylated handles and the 5'-ends of handles 1–6 are linked to the staples.

Staple extension name	Sequence
Biotinylated extensions	5'-/biotin/TTTTT-3'
For Dual-labeling:	
Handle **1** & **3**	5'-TTCCTCTACCACCTACATCAC-3'
Handle **2** & **5**	5'-TAACATTCCTAACTTCTCATA-3'
Handle **4**	5'-CTACCATCTCTCCTAAACTCA-3'
Handle **6**	5'-ACTCACCACCACAATATTATA-3'
For single-labeling:	
Handle **1** & **2** & **3** & **4**	5'-TTCCTCTACCACCTACATCAC-3'
Handle **5** & **6**	5'-TAACATTCCTAACTTCTCATA-3'
For DNA-PAINT:	
Handle **P1.1**, **P2.1**, **P3.1** & **P4.1**	5'-TTATACATCTA-3'
Handle **P1.2**, **P2.2**, **P3.2** & **P4.2**	5'-TTATCTACATA-3'
Handle **P1.3**, **P2.3**, **P3.3** & **P4.3**	5'-TTTCTTCATTA-3'

Table S.1.2 Handle sequences for three-point-star-like barcodes
Staple extension sequences used for constructing the three-point-star like nano-barcode. The staple extension names are colored to match the staples in the strand diagram shown in Figure S1b. Handles 1 and 2 are located at the ring and handles 3 and 4 are on the nanotube (Figure S1b). The 5'-ends of the handles are linked to the staples.

Staple extension name	Sequence
For fluorescent labeling:	
Handle **1**	5'-TTCCTCTACCACCTACATCAC-3'
Handle **3**	5'-TAACATTCCTAACTTCTCATA-3'
For linking nano-tubes to the ring:	
Handle **2**	5'-CTTCACACCACACTCCATCTA-3'
Handle **4**	5'-TAGATGGAGTGTGGTGTGAAG-3'

Table S.1.3 Anti-handle sequences for rod-like barcodes
Anti-handle sequences used for labeling rod-like barcodes. /AlexF488/ = Alexa Fluor 488; /AlexF647/ = Alexa Fluor 647. Colors refer to the pseudo-colors used in fluorescence imaging.

Anti-handle name	Color	Sequence
For Dual-labeling:		
Anti-handle **1** & **3**	Blue	5'-GTGATGTAGGTGGTAGAGGAA/AlexF488/-3'
	Green	5'-GTGATGTAGGTGGTAGAGGAA/Cy3/-3'
	Red	5'-GTGATGTAGGTGGTAGAGGAA/AlexF647/-3'
Anti-handle **2** & **5**	None	5'-TATGAGAAGTTAGGAATGTTA-3'
	Green	5'-TATGAGAAGTTAGGAATGTTA/Cy3/-3'
	Red	5'-TATGAGAAGTTAGGAATGTTA/AlexF647/-3'
Anti-handle **4**	None	5'-TGAGTTTAGGAGAGATGGTAG-3'
	Green	5'-TGAGTTTAGGAGAGATGGTAG/Cy3/-3'
	Red	5'-TGAGTTTAGGAGAGATGGTAG/AlexF647/-3'
Anti-handle **6**	Blue	5'-TATAATATTGTGGTGGTGAGT/AlexF488/-3'
	Green	5'-TATAATATTGTGGTGGTGAGT/Cy3/-3'
	Red	5'-TATAATATTGTGGTGGTGAGT/AlexF647/-3'
For single-labeling:		
Anti-handle **1** & **2** & **3** & **4**	Blue	5'-GTGATGTAGGTGGTAGAGGAA/AlexF488/-3'
	Green	5'-GTGATGTAGGTGGTAGAGGAA/Cy3/-3'
	Red	5'-GTGATGTAGGTGGTAGAGGAA/AlexF647/-3'
Anti-handle **5** & **6**	None	5'-TGAGTTTAGGAGAGATGGTAG-3'
	Green	5'-TATGAGAAGTTAGGAATGTTA/Cy3/-3'
	Red	5'-TATGAGAAGTTAGGAATGTTA /AlexF647/-3'
For DNA-PAINT:		
Anti-handle **P1.1, P2.1, P3.1 & P4.1**	Red	5'-CTAGATGTAT/ATTO655/-3'
Anti-handle **P1.2, P2.2, P3.2 & P4.2**	Green	5'-TATGTAGATC/Cy3b/-3'
Anti-handle **P1.3, P2.3, P3.3 & P4.3**	Blue	5'-GTAATGAAGA/ATTO488/-3'

Table S.1.4 Scaffold sequences

Scaffold sequences for six-helix-bundle ring (p3024) and nanotube (p7308).

P3024 sequence
CCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACGCGCGCTCACTGGCCGTCGTT
TTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCA
CATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCC
CAACAGTTGCGCAGCCTGAATGGCGAATGGGACGCGCCCTGTAGCGGCGCATTAAG
CGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAGC
GCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTC
AAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGA
CCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGAC
GGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAA
ACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGC
CGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATT
TTAACAAAATATTAACGCTTACAATTTAGGTGGCACTTTTCGGGGAAATGTGCGCGG
AACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAA
TAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACAT
TTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCA
GAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTA
CATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACG
TTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATT
GACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTT
GAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATT
ATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAAC
GATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC
TCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTG
ACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAAC
TACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTG
CAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGG
AGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCC
CTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAA
TAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCA
AGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCT
AGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTT
CCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTT
CTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGT
TTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCG
CAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAAC
TCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCA
GTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGG
CGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACG
ACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCC
GAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGC
GCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTC
GCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTAT
GGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGC
TCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTG
AGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGC
GAGGAAGCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGAT
TCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCA

ACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCT TCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAG CTATGACCATGATTACGCCAAGCGCGCAATTAACCCTCACTAAAGGGAACAAAAGCT GGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTGGATCCGTAAATCAATGACT TACGCGCACCGAAAGGTGCGTATTGTCTATAGCCCCCTCAGCCACGAATTCGTCTGA CGACGACAAGACAAGCTTGCGTGTGAATTCCCTGGCTTCTCCTGAGAAA
P7308 sequence
AATGCTACTACTATTAGTAGAATTGATGCCACCTTTTCAGCTCGCGCCCCAAATGAA AATATAGCTAAACAGGTTATTGACCATTTGCGAAATGTATCTAATGGTCAAACTAAA TCTACTCGTTCGCAGAATTGGGAATCAACTGTTATATGGAATGAAACTTCCAGACAC CGTACTTTAGTTGCATATTTAAAACATGTTGAGCTACAGCATTATATTCAGCAATTAA GCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGAGCAATTAAAGGTACTCT CTAATCCTGACCTGTTGGAGTTTGCTTCCGGTCTGGTTCGCTTTGAAGCTCGAATTAA AACGCGATATTTGAAGTCTTTCGGGCTTCCTCTTAATCTTTTTGATGCAATCCGCTTTG CTTCTGACTATAATAGTCAGGGTAAAGACCTGATTTTTGATTTATGGTCATTCTCGTT TTCTGAACTGTTTAAAGCATTTGAGGGGGATTCAATGAATATTTATGACGATTCCGCA GTATTGGACGCTATCCAGTCTAAACATTTTACTATTACCCCCTCTGGCAAAACTTCTT TTGCAAAAGCCTCTCGCTATTTTGGTTTTTATCGTCGTCTGGTAAACGAGGGTTATGA TAGTGTTGCTCTTACTATGCCTCGTAATTCCTTTTGGCGTTATGTATCTGCATTAGTTG AATGTGGTATTCCTAAATCTCAACTGATGAATCTTTCTACCTGTAATAATGTTGTTCC GTTAGTTCGTTTTATTAACGTAGATTTTTCTTCCCAACGTCCTGACTGGTATAATGAG CCAGTTCTTAAAATCGCATAAGGTAATTCACAATGATTAAAGTTGAAATTAAACCAT CTCAAGCCCAATTTACTACTCGTTCTGGTGTTTCTCGTCAGGGCAAGCCTTATTCACT GAATGAGCAGCTTTGTTACGTTGATTTGGGTAATGAATATCCGGTTCTTGTCAAGATT ACTCTTGATGAAGGTCAGCCAGCCTATGCGCCTGGTCTGTACACCGTTCATCTGTCCT CTTTCAAAGTTGGTCAGTTCGGTTCCCTTATGATTGACCGTCTGCGCCTCGTTCCGGC TAAGTAACATGGAGCAGGTCGCGGATTTCGACACAATTTATCAGGCGATGATACAAA TCTCCGTTGTACTTTGTTTCGCGCTTGGTATAATCGCTGGGGGTCAAAGATGAGTGTT TTAGTGTATTCTTTTGCCTCTTTCGTTTTAGGTTGGTGCCTTCGTAGTGGCATTACGTA TTTTACCCGTTTAATGGAAACTTCCTCATGAAAAAGTCTTTAGTCCTCAAAGCCTCTG TAGCCGTTGCTACCCTCGTTCCGATGCTGTCTTTCGCTGCTGAGGGTGACGATCCCGC AAAAGCGGCCTTTAACTCCCTGCAAGCCTCAGCGACCGAATATATCGGTTATGCGTG GGCGATGGTTGTTGTCATTGTCGGCGCAACTATCGGTATCAAGCTGTTTAAGAAATTC ACCTCGAAAGCAAGCTGATAAACCGATACAATTAAAGGCTCCTTTTGGAGCCTTTTT TTTGGAGATTTTCAACGTGAAAAAATTATTATTCGCAATTCCTTTAGTTGTTCCTTTCT ATTCTCACTCCGCTGAAACTGTTGAAAGTTGTTTAGCAAAATCCCATACAGAAAATT CATTTACTAACGTCTGGAAAGACGACAAAACTTTAGATCGTTACGCTAACTATGAGG GCTGTCTGTGGAATGCTACAGGCGTTGTAGTTTGTACTGGTGACGAAACTCAGTGTT ACGGTACATGGGTTCCTATTGGGCTTGCTATCCCTGAAAATGAGGGTGGTGGCTCTG AGGGTGGCGGTTCTGAGGGTGGCGGTTCTGAGGGTGGCGGTACTAAACCTCCTGAGT ACGGTGATACACCTATTCCGGGCTATACTTATATCAACCCTCTCGACGGCACTTATCC GCCTGGTACTGAGCAAAACCCCGCTAATCCTAATCCTTCTCTTGAGGAGTCTCAGCCT CTTAATACTTTCATGTTTCAGAATAATAGGTTCCGAAATAGGCAGGGGGCATTAACT GTTTATACGGGCACTGTTACTCAAGGCACTGACCCCGTTAAAACTTATTACCAGTAC ACTCCTGTATCATCAAAAGCCATGTATGACGCTTACTGGAACGGTAAATTCAGAGAC TGCGCTTTCCATTCTGGCTTTAATGAGGATTTATTTGTTTGTGAATATCAAGGCCAAT CGTCTGACCTGCCTCAACCTCCTGTCAATGCTGGCGGCGGCTCTGGTGGTGGTTCTGG TGGCGGCTCTGAGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGA GGGAGGCGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGC AAACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTCTG

ACGCTAAAGGCAAACTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCGATGGTTT
CATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGTGATTTTGCTGGC
TCTAATTCCCAAATGGCTCAAGTCGGTGACGGTGATAATTCACCTTTAATGAATAATT
TCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGAATGTCGCCCTTTTGTCTTTGGC
GCTGGTAAACCATATGAATTTTCTATTGATTGTGACAAAATAAACTTATTCCGTGGTG
TCTTTGCGTTTCTTTTATATGTTGCCACCTTTATGTATGTATTTTCTACGTTTGCTAACA
TACTGCGTAATAAGGAGTCTTAATCATGCCAGTTCTTTTGGGTATTCCGTTATTATTG
CGTTTCCTCGGTTTCCTTCTGGTAACTTTGTTCGGCTATCTGCTTACTTTTCTTAAAAA
GGGCTTCGGTAAGATAGCTATTGCTATTTCATTGTTTCTTGCTCTTATTATTGGGCTTA
ACTCAATTCTTGTGGGTTATCTCTCTGATATTAGCGCTCAATTACCCTCTGACTTTGTT
CAGGGTGTTCAGTTAATTCTCCCGTCTAATGCGCTTCCCTGTTTTTATGTTATTCTCTC
TGTAAAGGCTGCTATTTTCATTTTTGACGTTAAACAAAAAATCGTTTCTTATTTGGAT
TGGGATAAATAATATGGCTGTTTATTTTGTAACTGGCAAATTAGGCTCTGGAAAGAC
GCTCGTTAGCGTTGGTAAGATTCAGGATAAAATTGTAGCTGGGTGCAAAATAGCAAC
TAATCTTGATTTAAGGCTTCAAAACCTCCCGCAAGTCGGGAGGTTCGCTAAAACGCC
TCGCGTTCTTAGAATACCGGATAAGCCTTCTATATCTGATTTGCTTGCTATTGGGCGC
GGTAATGATTCCTACGATGAAAATAAAAACGGCTTGCTTGTTCTCGATGAGTGCGGT
ACTTGGTTTAATACCCGTTCTTGGAATGATAAGGAAAGACAGCCGATTATTGATTGG
TTTCTACATGCTCGTAAATTAGGATGGGATATTATTTTTCTTGTTCAGGACTTATCTAT
TGTTGATAAACAGGCGCGTTCTGCATTAGCTGAACATGTTGTTTATTGTCGTCGTCTG
GACAGAATTACTTTACCTTTTGTCGGTACTTTATATTCTCTTATTACTGGCTCGAAAAT
GCCTCTGCCTAAATTACATGTTGGCGTTGTTAAATATGGCGATTCTCAATTAAGCCCT
ACTGTTGAGCGTTGGCTTTATACTGGTAAGAATTTGTATAACGCATATGATACTAAAC
AGGCTTTTTCTAGTAATTATGATTCCGGTGTTTATTCTTATTTAACGCCTTATTTATCA
CACGGTCGGTATTTCAAACCATTAAATTTAGGTCAGAAGATGAAATTAACTAAAATA
TATTTGAAAAAGTTTTCTCGCGTTCTTTGTCTTGCGATTGGATTTGCATCAGCATTTAC
ATATAGTTATATAACCCAACCTAAGCCGGAGGTTAAAAAGGTAGTCTCTCAGACCTA
TGATTTTGATAAATTCACTATTGACTCTTCTCAGCGTCTTAATCTAAGCTATCGCTAT
GTTTTCAAGGATTCTAAGGGAAAATTAATTAATAGCGACGATTTACAGAAGCAAGGT
TATTCACTCACATATATTGATTTATGTACTGTTTCCATTAAAAAAGGTAATTCAAATG
AAATTGTTAAATGTAATTAATTTTGTTTTCTTGATGTTTGTTTCATCATCTTCTTTTGCT
CAGGTAATTGAAATGAATAATTCGCCTCTGCGCGATTTTGTAACTTGGTATTCAAAGC
AATCAGGCGAATCCGTTATTGTTTCTCCCGATGTAAAAGGTACTGTTACTGTATATTC
ATCTGACGTTAAACCTGAAAATCTACGCAATTTCTTTATTTCTGTTTTACGTGCAAAT
AATTTTGATATGGTAGGTTCTAACCCTTCCATTATTCAGAAGTATAATCCAAACAATC
AGGATTATATTGATGAATTGCCATCATCTGATAATCAGGAATATGATGATAATTCCG
CTCCTTCTGGTGGTTTCTTTGTTCCGCAAAATGATAATGTTACTCAAACTTTTAAAATT
AATAACGTTCGGGCAAAGGATTTAATACGAGTTGTCGAATTGTTTGTAAAGTCTAAT
ACTTCTAAATCCTCAAATGTATTATCTATTGACGGCTCTAATCTATTAGTTGTTAGTG
CTCCTAAAGATATTTTAGATAACCTTCCTCAATTCCTTTCAACTGTTGATTTGCCAACT
GACCAGATATTGATTGAGGGTTTGATATTTGAGGTTCAGCAAGGTGATGCTTTAGATT
TTTCATTTGCTGCTGGCTCTCAGCGTGGCACTGTTGCAGGCGGTGTTAATACTGACCG
CCTCACCTCTGTTTTATCTTCTGCTGGTGGTTCGTTCGGTATTTTTAATGGCGATGTTT
TAGGGCTATCAGTTCGCGCATTAAAGACTAATAGCCATTCAAAAATATTGTCTGTGC
CACGTATTCTTACGCTTTCAGGTCAGAAGGGTTCTATCTCTGTTGGCCAGAATGTCCC
TTTTATTACTGGTCGTGTGACTGGTGAATCTGCCAATGTAAATAATCCATTTCAGACG
ATTGAGCGTCAAAATGTAGGTATTTCCATGAGCGTTTTTCCTGTTGCAATGGCTGGCG
GTAATATTGTTCTGGATATTACCAGCAAGGCCGATAGTTTGAGTTCTTCTACTCAGGC
AAGTGATGTTATTACTAATCAAAGAAGTATTGCTACAACGGTTAATTTGCGTGATGG
ACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAACACTTCTCAGGATTCTGGC
GTACCGTTCCTGTCTAAAATCCCTTTAATCGGCCTCCTGTTTAGCTCCCGCTCTGATTC

TAACGAGGAAAGCACGTTATACGTGCTCGTCAAAGCAACCATAGTACGCGCCCTGTA
GCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCGCTACACTTG
CCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCC
GGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTT
TACGGCACCTCGACCCCAAAAAACTTGATTTGGGTGATGGTTCACGTAGTGGGCCAT
CGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGG
ACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGGCTATTCTTTTGATTTA
TAAGGGATTTTGCCGATTTCGGAACCACCATCAAACAGGATTTTCGCCTGCTGGGGC
AAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATC
AGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAA
CCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCG
ACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGG
CACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGG
ATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGAATTCGAGCTCGGTAC
CCGGGGATCCTTATACGGGTACTAGCCATGCGTATACGGTCGCTAGCGGACTTGCCT
CGCTATCAAAGGTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGT
TTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGC
ACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTC
CCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGA
AGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGT
CCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTA
TCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCG
CTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTT
GATGGCGTTCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAATGCGAATT
TTAACAAAATATTAACGTTTACAATTTAAATATTTGCTTATACAATCTTCCTGTTTTTG
GGGCTTTTCTGATTATCAACCGGGGTACATATGATTGACATGCTAGTTTTACGATTAC
CGTTCATCGATTCTCTTGTTTGCTCCAGACTCTCAGGCAATGACCTGATAGCCTTTGT
AGATCTCTCAAAAATAGCTACCCTCTCCGGCATTAATTTATCAGCTAGAACGGTTGA
ATATCATATTGATGGTGATTTGACTGTCTCCGGCCTTTCTCACCCTTTTGAATCTTTAC
CTACACATTACTCAGGCATTGCATTTAAAATATATGAGGGTTCTAAAAATTTTTATCC
TTGCGTTGAAATAAAGGCTTCTCCCGCAAAAGTATTACAGGGTCATAATGTTTTTGGT
ACAACCGATTTAGCTTTATGCTCTGAGGCTTTATTGCTTAATTTTGCTAATTCTTTGCC
TTGCCTGTATGATTTATTGGATGTT

Table S.1.5 Barcode geometry measurement results

Results of automated barcode geometry measurement by analyzing one $71\times71\ \mu m^2$ image for each barcode species. The geometrical parameters are listed as $(mean \pm stdev)$. Each pixel is equal to 71 nm.

Barcode type	Barcode identified	Bending angle α (degree)	Larger distance (pixel)	Smaller distance (pixel)
BRG	70	165.11±10.08	6.18±0.76	3.77±0.74
GRG	81	161.51±11.73	6.12±0.95	3.95±0.98

Table S.1.6 Decoding result of the 72-barcode pool
Computer aided barcode-counting result for the 72-barcode-species mixture. The barcodes are sorted by counts (from highest to lowest). The barcode species that were expected in the mixture are highlighted. In total 2,556 of the 2,617 registered barcodes are the expected species. On average there are 35.5 ± 8.2 $(mean \pm stdev)$ barcodes per expected species and only 1.4 ± 0.7 $(mean \pm stdev)$ barcodes per false positive species.

Barcode name	Counts	Barcode name	Counts
blue--blue-red	50	blue--red-green	50
red--green-blue	49	blue--green/blue-red	48
green/blue--blue-green	48	green/blue--red-green/blue	48
green/blue--red/blue-green	47	blue--blue-green	46
green/blue--green-blue	45	red--blue-green	45
blue--green-blue	44	green--green-blue	44
red/blue--red-green	44	green/blue--blue-red/green	43
red/green--red-blue	43	green--green/blue-red	42
red/blue--red/blue-green	42	red/green--blue-red/green	42
green--blue-red	41	green--blue-red/green	41
red/blue--green-red/blue	41	red/green--green-blue	41
red/green--green-red/blue	41	blue--red-green/blue	40
green--blue-green	40	red--green-red/blue	40
blue--red/blue-green	39	blue--red-blue	38
red/blue--blue-red	38	red/green--red/blue-green	38
red/blue--blue-green	37	red/blue--red/green-blue	37
red/green--blue-red	36	blue--green-red	35
blue--green-red/blue	35	green--green-red/blue	35
green--red-green	35	green--red/blue-green	35
green/blue--red-blue	35	red--red/blue-green	35
red/green--red-green/blue	35	green--green-red	34
red/blue--blue-red/green	34	red/blue--green-blue	34
green--red-blue	33	green--red-green/blue	33
red--green-red	33	red--red/green-blue	33
red/green--green/blue-red	33	green/blue--green-red/blue	32
blue--red/green-blue	31	green/blue--green/blue-red	31
green/blue--red-green	31	red/blue--red-green/blue	31
red/green--red/green-blue	31	green/blue--red/green-blue	29
red/green--blue-green	29	red--green/blue-red	28
red--red-blue	28	green/blue--blue-red	27
red--red-green	27	red--red-green/blue	27
blue--blue-red/green	26	red/green--green-red	26
green/blue--green-red	24	red--blue-red	24
red/blue--green/blue-red	24	red/green--red-green	23

green--red/green-blue	21	red--blue-red/green	19
red/blue--red-blue	17	red/blue--green-red	15
green--red/blue-red	4	blue--red/blue-green/blue	3
red/blue--red/blue-blue	3	blue--green/blue-red/green	2
blue--red/green-green	2	blue--red/green-green/blue	2
green--red/green-red/blue	2	green/blue--green/blue-red/green	2
green/blue--red/green-green/blue	2	red--green/blue-green	2
red/blue--green/blue-red/green	2	red/green--red/blue-blue	2
red/green--red/green-red/blue	2	blue--blue-blue	1
blue--green-green/blue	1	blue--green-red/green	1
blue--green/blue-blue	1	blue--green/blue-red/blue	1
blue--red/blue-blue	1	green--green/blue-red/green	1
green--red-red/blue	1	green--red/green-green/blue	1
green--red/green-red	1	green/blue--green-green/blue	1
green/blue--red-red/blue	1	green/blue--red/blue-blue	1
green/blue--red/blue-red/green	1	green/blue--red/green-red/blue	1
red--blue-green/blue	1	red--green-red/green	1
red--red/blue-green/blue	1	red--red/green-red/blue	1
red/blue--blue-green/blue	1	red/blue--green-green/blue	1
red/blue--green/blue-blue	1	red/blue--green/blue-green	1
red/blue--red-red/blue	1	red/blue--red-red/green	1
red/blue--red/blue-red/green	1	red/blue--red/green-red/blue	1
red/green--green/blue-blue	1	red/green--green/blue-green	1
red/green--green/blue-red/blue	1	red/green--red/green-red	1

Table S.1.7 Decoding result of the 216-barcode pool
Computer aided barcode-counting result for the 216-barcode-species mixture. The barcodes are sorted by counts (from highest to lowest). In total 7,243 qualified barcodes were registered. On average there are 33.5 ± 16.7 $(mean \pm stdev)$ barcodes per species.

Barcode name	Counts	Barcode name	Counts
blue--green-green	114	blue--blue-blue	102
green--blue-blue	99	blue--green-blue	95
blue--blue-green	86	green--green-blue	81
green--blue-green	81	green--green-green	71
blue--green/blue-green	71	green/blue--green-green	70
green/blue--blue-blue	70	green--red/blue-red	69
red--green-blue	63	blue--red/green-red/green	62
blue--red/green-red	61	blue--green/blue-blue	61
green/blue--blue-green	59	green--red-blue	57
red/green--blue-blue	57	red--green-green	55
red/blue--blue-green	55	red/blue--green-green	54
red--blue-blue	53	blue--red-red	52
blue--green/blue-red	51	blue--red-green	50
blue--red/green-green	50	red/green--green-blue	50
blue--green-red	49	red/blue--green/blue-green	48
green--red/green-blue	47	red/blue--red/blue-green	46
green/blue--green-blue	46	green/blue--green/blue-green	45
red--blue-green	43	blue--blue-red	43
green/blue--green/blue-red	43	green--green/blue-red	42
red/green--red/blue-red	42	red/blue--blue-blue	42
green/blue--red/blue-red/green	42	green--green-red	41
green--red/blue-blue	41	green--green/blue-green	41
blue--red-red/green	41	blue--red/blue-red	41
blue--red/blue-red/green	41	red/green--green/blue-blue	41
red/blue--green-blue	41	red/blue--red/green-red	41
red/blue--red/blue-red	41	green/blue--blue-red	41
green--green/blue-blue	40	green--blue-red	39
blue--red/green-blue	39	red/green--green/blue-red/blue	39
red--red/green-blue	38	red--green/blue-green	38
green--red/green-red/blue	38	red/blue--green/blue-blue	38
green/blue--red/green-green	38	red--red/blue-red	37
red--green/blue-blue	37	green--blue-red/green	37
blue--green/blue-green/blue	37	green/blue--green-red/green	37
green--red-red	36	red/green--green-green	36
red/blue--green/blue-red	36	green/blue--red-red	36

green/blue--red-red/green	36	red--red/blue-green	35
blue--green-red/green	35	blue--red/green-red/blue	35
red/green--red/blue-red/blue	35	green/blue--red/green-red	35
green--red/green-red	34	green--green/blue-red/blue	34
green--red/green-green	33	blue--green/blue-red/green	33
blue--green/blue-red/blue	33	red/green--blue-green	33
red/green--red/blue-blue	33	red/green--green/blue-green	33
green/blue--red/blue-blue	33	green/blue--green/blue-red/green	33
green--red-red/blue	32	green--blue-red/blue	32
blue--red-blue	32	blue--red/blue-green	32
red/blue--red/green-green	32	green/blue--red/green-red/green	32
blue--red-green/blue	31	red/blue--blue-red/green	31
green/blue--red-red/blue	31	green/blue--blue-red/green	31
red--green-green/blue	30	green--green-red/blue	30
red/green--red-blue	30	red/green--red-red/blue	30
red/green--blue-red	30	red/blue--red-green	30
red/blue--green-red	30	green/blue--green/blue-blue	30
red--red-green	29	red--blue-red	29
blue--blue-red/green	29	blue--red/green-green/blue	29
blue--red/blue-green/blue	29	red/green--red-red	29
red/green--green-red	29	red/blue--red/blue-red/green	29
red/blue--green/blue-red/blue	29	green/blue--red/green-blue	29
green/blue--red/green-red/blue	29	green/blue--red/green-green/blue	29
green/blue--green/blue-red/blue	29	red--red/green-red/blue	28
green--red/green-red/green	28	green--red/blue-green	28
green--red/blue-red/blue	28	red/blue--red-red	28
green/blue--green/blue-green/blue	28	green--red/blue-red/green	27
blue--green-green/blue	27	blue--blue-green/blue	27
red/green--blue-green/blue	27	red/green--red/green-red/blue	27
red/blue--green-green/blue	27	red/blue--blue-red	27
red/blue--blue-green/blue	27	red/blue--red/green-blue	27
red/blue--red/blue-blue	27	green/blue--red-green/blue	27
red--red-red/green	26	red--green-red	26
red--red/green-green/blue	26	red--green/blue-green/blue	26
green--green-red/green	26	green--green/blue-green/blue	26
blue--red/blue-red/blue	26	red/green--green/blue-red	26
red/blue--green/blue-green/blue	26	green/blue--red-green	26
red--red-red	25	red--red-blue	25
red--blue-green/blue	25	red--red/blue-green/blue	25
green--red-green	25	red/green--blue-red/blue	25
red/green--red/green-blue	25	red/green--green/blue-green/blue	25

green/blue--red-blue	25	green/blue--green-red	25
green/blue--green-red/blue	25	green/blue--blue-green/blue	25
green/blue--red/blue-red	25	green/blue--red/blue-green	25
red--red/green-green	24	green--green-green/blue	24
green/blue--red/blue-red/blue	24	red--red/blue-blue	23
red--red/blue-red/green	23	red--green/blue-red	23
red/green--red/green-green	23	red/blue--red/green-red/blue	23
green/blue--green-green/blue	23	red--green-red/blue	22
green--red-green/blue	22	blue--green-red/blue	22
red/green--red/blue-green/blue	22	red/blue--green-red/green	22
red/blue--red/green-red/green	22	green/blue--blue-red/blue	22
green/blue--red/blue-green/blue	22	red--blue-red/green	21
green--blue-green/blue	21	green--green/blue-red/green	21
red/green--red-green/blue	21	red/green--blue-red/green	21
red/green--red/green-red	20	red/green--red/blue-green	20
red/blue--red/blue-green/blue	20	red/blue--green/blue-red/green	20
red--red-green/blue	19	red--red/green-red/green	19
blue--red/blue-blue	19	red/green--green/blue-red/green	19
red/blue--red-red/green	19	red/blue--red-red/blue	19
red/green--red/blue-red/green	18	red/blue--blue-red/blue	18
red--blue-red/blue	17	blue--blue-red/blue	17
red/green--green-red/blue	17	red/green--red/green-green/blue	17
red/blue--red/green-green/blue	17	red--green-red/green	16
red--red/green-red	16	red--red/blue-red/blue	16
green--red-red/green	16	green--red/blue-green/blue	16
red/blue--green-red/blue	16	red/blue--red/blue-red/blue	16
red--red-red/blue	15	red--green/blue-red/blue	15
green--red/green-green/blue	15	blue--red-red/blue	15
red/green--red-red/green	15	red/green--green-green/blue	15
red/blue--red-blue	15	red--green/blue-red/green	14
red/green--red-green	13	red/green--green-red/green	13
red/blue--red-green/blue	13	red/green--red/green-red/green	12

Supplementary figure captions

Figure S.1.1 Strand diagram of barcode design. The strands are colored according to the staple extensions in table S1. (a) 800-nm-tube-based barcode (b) star-like barcode. (c) 400 nm super-resolution barcode. (d) Single-layer DNA origami marker for super-resolution drift correction and alignment.

Figure S.1.2 Representative epi-fluorescence microscope image of BRG nano-barcode. Scale bar: 5 μm.

Figure S.1.3 Representative TIRF microscope images of the selected 5 single-labeled-zone nano-barcode. Scale bar: 5 μm.

Figure S.1.4 Diagrams showing the automated barcode geometry measurement. (a) TIRF microscope image of BRG barcode. (b) The same image after computer processing. The centroid of each fluorescent spot is shown as one square (pixel). Inset: a schematic diagram to illustrate the distances and angles measured by the software to characterize the barcode geometry. (c) TIRF microscope image of GRG barcode. (d) The same image after computer processing. Scale bar: 10 pixel = 710 nm.

Figure S.1.5 Representative TEM image of the 800-nm-tube-based barcodes.

Figure S.1.6 Representative TIRF microscope images of 1:1 mixture of BRG and RGB barcode. Note the false-positive barcode highlighted by a yellow $1.4\times0.7\ \mu m^2$ rectangle and zoomed-in 5× in the inset. Scale bar: 5 μm.

Figure S.1.7 Representative TIRF microscope images of an equimolar mixture of all 27 single-labeled-zone barcode family members. Scale bar: 5 μm.

Figure S.1.8 Representative TIRF microscope images of the selected 5 dual-labeled-zone nano-barcode. Scale bar: 5 μm.

Figure S.1.9 Representative TIRF microscope images of an equimolar mixture of the selected 72 dual-labeled-zone barcode family members. Scale bar: 5 μm.

Figure S.1.10 Block diagram showing the principle of automated decoding software.

Figure S.1.11 Statistics obtained by analyzing mixtures of (a) 72 barcodes in supervised mode, (b) 72 barcodes in unsupervised mode, and (c) 216 barcodes in unsupervised mode. The barcode counts are listed in table S6 and S7. The barcode types are shown as computer-generated "ideal" images and placed to the left of the corresponding bar graphs. The barcodes are sorted in a descending order of counts. Annotations above the bar graphs were used to denote the expected and unexpected species in (a) and (b), with one exception in (b) where an expected barcode (BR--B-R) was denoted by *.

Figure S.1.12 Representative TIRF microscope images of an equimolar mixture of all 216 dual-labeled-zone barcode family members. Scale bar: 5 μm.

Figure S.1.13 Diffraction-limited and super-resolution overview images of 800 nm DNA origami-based barcodes. (a) Diffraction-limited image of nano-barcodes, statically labeled with Alexa488 together with a super-resolution reconstruction overlay obtained using DNA-PAINT. (b) Diffraction-limited image of the same sample region using DNA-

PAINT and overlaid super-resolution reconstruction. Scale bars: 2 μm. The nano-barcodes are specifically imaged with a high efficiency using DNA-PAINT. Some parts of the Alexa488 labeled nano-barcodes are already bleached or labeled with inactive fluorophores (see red highlighted area), but are correctly imaged using DNA-PAINT. The few non-specific spots in the DNA-PAINT image can be ruled out by investigating the binding frequency pattern, i.e. non-specific binding per area happens with a lower probability or even only once during imaging acquisition (cf. intensity vs. time plots for the grey (non-specific binding) and green (specific binding) highlighted regions).

Figure S.1.14 Super-resolved DNA-PAINT zoomed-out image. Scale bar: 500 nm.

Figure S.1.15 Representative TIRF microscope images of the three-point-star-like barcodes. Scale bar: 10 μm.

Figure S.1.16 Representative TEM images of the three-point-star-like barcodes.

Figure S.1.17 Representative TIRF images showing the GRG-barcode-treated *C. albicans* cells. (a) GRG-barcode-tagged *C. albicans* cells (scattered). (b) GRG-barcode-tagged *C. albicans* cells (aggregated). (c) *C. albicans* cells treated with non-biotinylated antibodies. (d) *C. albicans* cells treated with non-biotinylated GRG barcodes. Scale bar: 5 μm.

handle 1 handle 2

Front monomer of 6hb nanotube for 800-nm barcode

handle 3 handle 4 handle 5 handle 6

Rare monomer of 6hb nanotube for 800-nm barcode

Fig S.1.1 (a) (Zoom in to see the details)

6hb ring for 3-point-like barcode

6hb nanotube for 3-point-like barcode

Fig S.1.1 (b) (Zoom in to see the details)

Fig S.1.1 (c) (Zoom in to see the details)

Fig S1.1 (d) (Zoom in to see the detail)

Figure S.1.2

Figure S.1.3 (Zoom in to see the details)

Figure S.1.4

Figure S.1.5

Figure S.1.6

Figure S.1.7 (Zoom in to see the details)

Figure S.1.8 (Zoom in to see the details)

Figure S.1.9 (Zoom in to see the details)

Step 1:
Locate Barcodes
Preprocessing
• High Pass Spatial Filter
• Threshold
• Merge color channels
Locate Barcodes
Step 2:
Read Barcode
For Each Located Barcode
Reference Library
1
2
3
4
5
6
215
216
Matched Filter
Most Likely Match
(BG -- BR - R)
(Optional)
Prompt
User to
Accept or Override

Figure S.1.10

Figure S.1.11

Figure S.1.12 (Zoom in to see the details)

a
b
binding event
1
0
0
100
200
300
400
500
time (s)
binding event
1
0
0
100
200
300
400
500
time (s)

Figure S.1.13

Fig S.1.14 (Zoom in to see the details)

Figure S.1.15

Figure S.1.16

Figure S.1.17