

Tutorial for cell nucleus detection and counting on histochemically stained tissue microarray (TMA) images with Color Deconvolution.

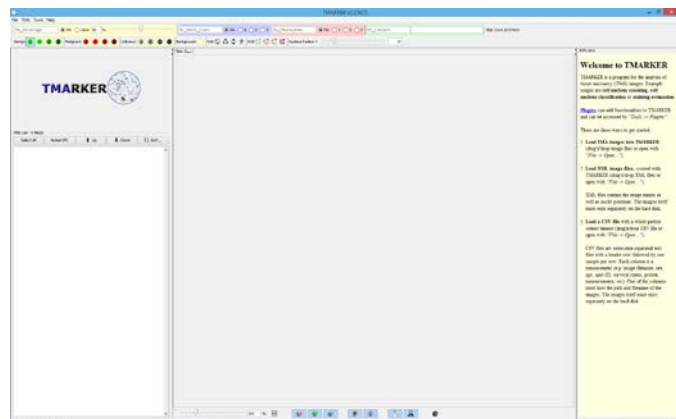
<http://www.nexus.ethz.ch/> -> Software -> TMARKER

PREREQUISITES:

- Java 1.7 (Runtime Environment JRE).
- Tissue images of IHC stained tissue (see DemonstrationData.zip)

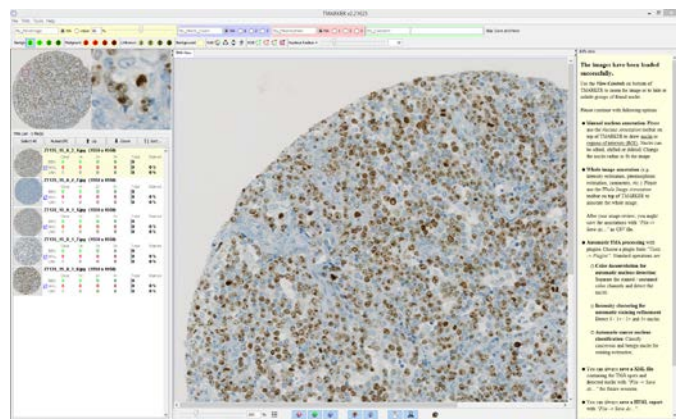
WORKFLOW

1. Open TMARKER.

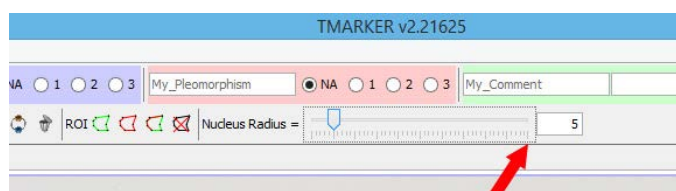


2. Drag and Drop the images (one image per TMA spot) from the demonstration dataset (Folder "RCC") into TMARKER.

(Or alternatively click "File -> Open..." and select the images).



3. Set the nucleus radius to 5.



4. Select all images that you want to process with nucleus detection.

TMA List - 5 file(s)

Select All Human/PC Up Down Sort...

Image	Clear	1+	2+	3+	Total	Stained
ZT135_15_A_2_1.jpg (1550 x 1550)	BEN 0	0	0	0	0	0 %
	MAL 0	0	0	0	0	0 %
	UNK 0	0	0	0	0	0 %
ZT135_15_A_4_7.jpg (1550 x 1550)	BEN 0	0	0	0	0	0 %
	MAL 0	0	0	0	0	0 %
	UNK 0	0	0	0	0	0 %
ZT135_15_A_1_1.jpg (1550 x 1550)	BEN 0	0	0	0	0	0 %
	MAL 0	0	0	0	0	0 %
	UNK 0	0	0	0	0	0 %
ZT135_15_A_1_7.jpg (1550 x 1550)	BEN 0	0	0	0	0	0 %
	MAL 0	0	0	0	0	0 %
	UNK 0	0	0	0	0	0 %

5. Open the “Color Deconvolution” plugin in the tools and use following parameters in the upcoming window:

Staining Protocol = H DAB.
 Tolerance = 5
 Blur = 2
 T_hema = 55
 T_dab = 110

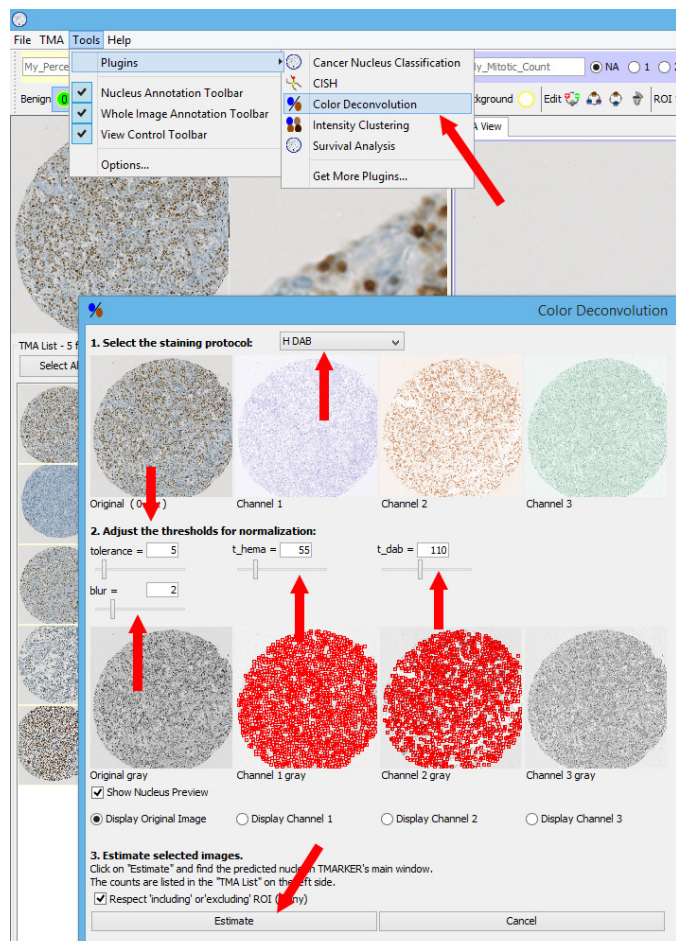
Click on „Estimate“.

This is the **Color-Deconvolution** method. The two color channel images Hema and DAB are separated. They can be seen by selecting “Display Channel 1” or “2” in the plugin after click on “Estimate”.

How are nuclei detected?

The two channels are blurred and local intensity minima are detected in the image. A local minimum is accepted if it is below the threshold and if it has a minimum difference to the neighboring local minimum of *tolerance*.

Accepted nuclei are filtered not to overlap to each other according to their radius.



Repeat step 5 with different parameters until you are satisfied with the result. TMARKER gives immediate feedback in the preview images and recalculates fast the detected nuclei.

You might want to select the optimal parameters on one image and then process all images.

6. The nuclei are displayed in the main window as “**Unknown**” nuclei (since they do not have a known cancer/benign state – they are just detected).

As the nuclei arise from the *Hema* (unstained) channel and from the *DAB* (stained) channel, they appear in the “**Clear**” and “**3+**” column of the TMA List.

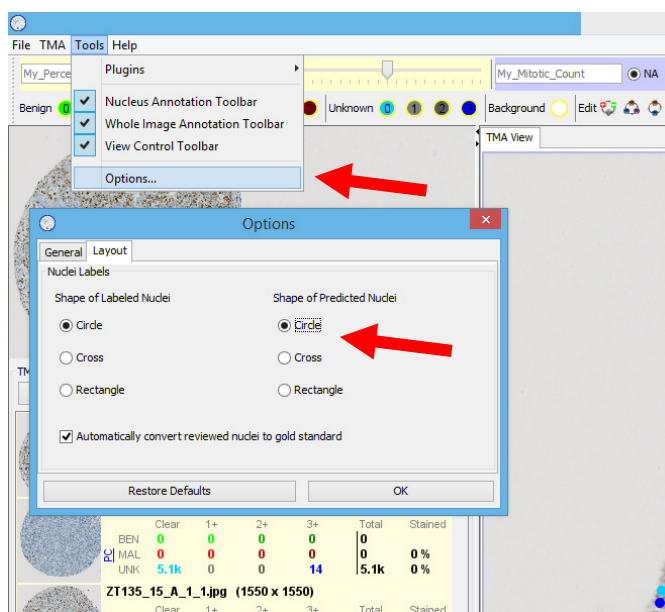
Example here: 2.2k blue nuclei (unstained) and 2.6k brown nuclei (stained) have been found (54% stained).

TMA List - 5 file(s)

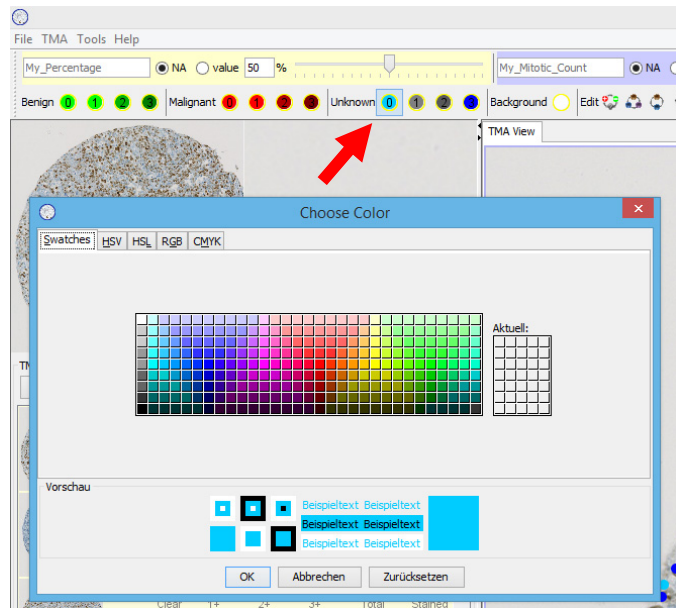
	Clear	1+	2+	3+	Total	Stained
ZT135_15_A_2_1.jpg (1550 x 1550)						
BEN	0	0	0	0	0	0 %
MAL	0	0	0	0	0	0 %
UNK	2.2k	0	0	2.6k	4.8k	54 %
ZT135_15_A_4_7.jpg (1550 x 1550)						
BEN	0	0	0	0	0	0 %
MAL	0	0	0	0	0	0 %
UNK	5.1k	0	0	14	5.1k	0 %
ZT135_15_A_1_1.jpg (1550 x 1550)						
BEN	0	0	0	0	0	0 %
MAL	0	0	0	0	0	0 %
UNK	2.6k	0	0	2.0k	4.7k	43 %
ZT135_15_A_1_7.jpg (1550 x 1550)						
BEN	0	0	0	0	0	0 %
MAL	3.7k	0	0	510	4.2k	12 %
UNK	1.7k	0	0	2.8k	4.5k	62 %
ZT135_15_A_1_8.jpg (1550 x 1550)						
BEN	0	0	0	0	0	0 %
MAL	0	0	0	0	0	0 %
UNK	1.7k	0	0	2.8k	4.5k	62 %

TIP: You might want to adjust the shape and the color of the nuclei for better visualization:

Shape: Click on *Tools -> Options -> Layout* and select “Circle”.



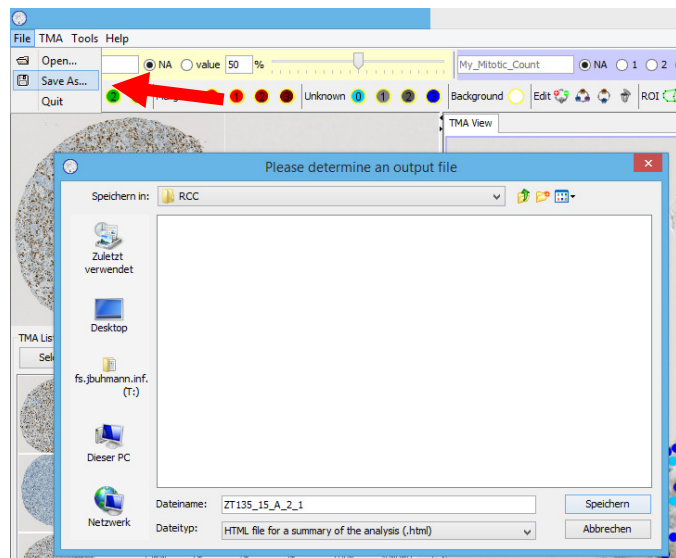
Color: Double click on the *Unknown* Label and select the color.

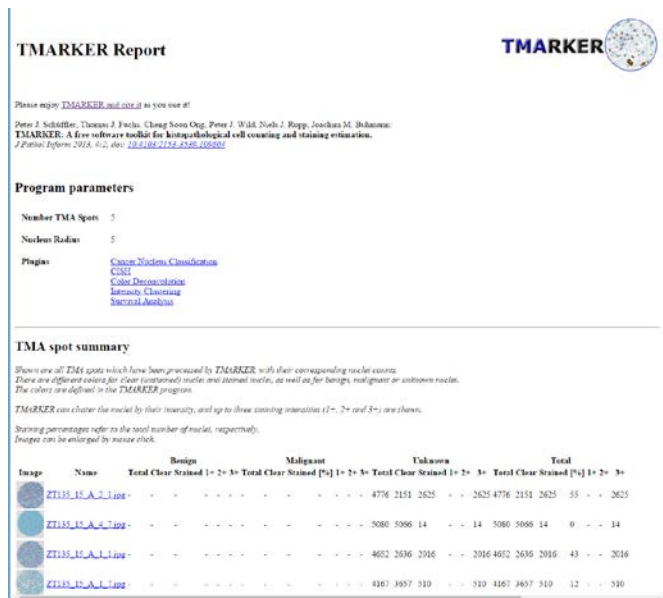


7. Save the analysis output.

- A) As a HTML Report. Go to “*File -> Save As...*” and select a new HTML file.

A HTML report with a sub-folder for the needed image files is created.





- B) Save the analysis as XML which can be reloaded in future TMARKER sessions.

The stored XML file contains all program options and all TMA spots and their nuclei. The file can be opened (or drag'n'dropped) in the next TMARKER session.

The raw images have to co-exist on the hard disk (they are not included in the XML file itself).

```

ZT135_15_A_2.xml x
1 100
2 100
3 100
4 100
5 100
6 100
7 100
8 100
9 100
10 100
11 100
12 100
13 100
14 100
15 100
16 100
17 100
18 100
19 100
20 100
21 100
22 100
23 100
24 100
25 100
26 100
27 100
28 100
29 100
30 100
31 100
32 100
33 100
34 100
35 100
36 100
37 100
38 100
39 100
40 100
41 100
42 100
43 100
44 100
45 100
46 100
47 100
48 100
49 100
50 100
51 100
52 100
53 100
54 100
55 100
56 100
57 100
58 100
59 100
60 100
61 100
62 100
63 100
64 100
65 100
66 100
67 100
68 100
69 100
70 100
71 100
72 100
73 100
74 100
75 100
76 100
77 100
78 100
79 100
80 100
81 100
82 100
83 100
84 100
85 100
86 100
87 100
88 100
89 100
90 100
91 100
92 100
93 100
94 100
95 100
96 100
97 100
98 100
99 100
100 100
101 100
102 100
103 100
104 100
105 100
106 100
107 100
108 100
109 100
110 100
111 100
112 100
113 100
114 100
115 100
116 100
117 100
118 100
119 100
120 100
121 100
122 100
123 100
124 100
125 100
126 100
127 100
128 100
129 100
130 100
131 100
132 100
133 100
134 100
135 100
136 100
137 100
138 100
139 100
140 100
141 100
142 100
143 100
144 100
145 100
146 100
147 100
148 100
149 100
150 100
151 100
152 100
153 100
154 100
155 100
156 100
157 100
158 100
159 100
160 100
161 100
162 100
163 100
164 100
165 100
166 100
167 100
168 100
169 100
170 100
171 100
172 100
173 100
174 100
175 100
176 100
177 100
178 100
179 100
180 100
181 100
182 100
183 100
184 100
185 100
186 100
187 100
188 100
189 100
190 100
191 100
192 100
193 100
194 100
195 100
196 100
197 100
198 100
199 100
200 100
201 100
202 100
203 100
204 100
205 100
206 100
207 100
208 100
209 100
210 100
211 100
212 100
213 100
214 100
215 100
216 100
217 100
218 100
219 100
220 100
221 100
222 100
223 100
224 100
225 100
226 100
227 100
228 100
229 100
230 100
231 100
232 100
233 100
234 100
235 100
236 100
237 100
238 100
239 100
240 100
241 100
242 100
243 100
244 100
245 100
246 100
247 100
248 100
249 100
250 100
251 100
252 100
253 100
254 100
255 100
256 100
257 100
258 100
259 100
260 100
261 100
262 100
263 100
264 100
265 100
266 100
267 100
268 100
269 100
270 100
271 100
272 100
273 100
274 100
275 100
276 100
277 100
278 100
279 100
280 100
281 100
282 100
283 100
284 100
285 100
286 100
287 100
288 100
289 100
290 100
291 100
292 100
293 100
294 100
295 100
296 100
297 100
298 100
299 100
300 100
301 100
302 100
303 100
304 100
305 100
306 100
307 100
308 100
309 100
310 100
311 100
312 100
313 100
314 100
315 100
316 100
317 100
318 100
319 100
320 100
321 100
322 100
323 100
324 100
325 100
326 100
327 100
328 100
329 100
330 100
331 100
332 100
333 100
334 100
335 100
336 100
337 100
338 100
339 100
340 100
341 100
342 100
343 100
344 100
345 100
346 100
347 100
348 100
349 100
350 100
351 100
352 100
353 100
354 100
355 100
356 100
357 100
358 100
359 100
360 100
361 100
362 100
363 100
364 100
365 100
366 100
367 100
368 100
369 100
370 100
371 100
372 100
373 100
374 100
375 100
376 100
377 100
378 100
379 100
380 100
381 100
382 100
383 100
384 100
385 100
386 100
387 100
388 100
389 100
390 100
391 100
392 100
393 100
394 100
395 100
396 100
397 100
398 100
399 100
400 100
401 100
402 100
403 100
404 100
405 100
406 100
407 100
408 100
409 100
410 100
411 100
412 100
413 100
414 100
415 100
416 100
417 100
418 100
419 100
420 100
421 100
422 100
423 100
424 100
425 100
426 100
427 100
428 100
429 100
430 100
431 100
432 100
433 100
434 100
435 100
436 100
437 100
438 100
439 100
440 100
441 100
442 100
443 100
444 100
445 100
446 100
447 100
448 100
449 100
450 100
451 100
452 100
453 100
454 100
455 100
456 100
457 100
458 100
459 100
460 100
461 100
462 100
463 100
464 100
465 100
466 100
467 100
468 100
469 100
470 100
471 100
472 100
473 100
474 100
475 100
476 100
477 100
478 100
479 100
480 100
481 100
482 100
483 100
484 100
485 100
486 100
487 100
488 100
489 100
490 100
491 100
492 100
493 100
494 100
495 100
496 100
497 100
498 100
499 100
500 100
501 100
502 100
503 100
504 100
505 100
506 100
507 100
508 100
509 100
510 100
511 100
512 100
513 100
514 100
515 100
516 100
517 100
518 100
519 100
520 100
521 100
522 100
523 100
524 100
525 100
526 100
527 100
528 100
529 100
530 100
531 100
532 100
533 100
534 100
535 100
536 100
537 100
538 100
539 100
540 100
541 100
542 100
543 100
544 100
545 100
546 100
547 100
548 100
549 100
550 100
551 100
552 100
553 100
554 100
555 100
556 100
557 100
558 100
559 100
560 100
561 100
562 100
563 100
564 100
565 100
566 100
567 100
568 100
569 100
570 100
571 100
572 100
573 100
574 100
575 100
576 100
577 100
578 100
579 100
580 100
581 100
582 100
583 100
584 100
585 100
586 100
587 100
588 100
589 100
590 100
591 100
592 100
593 100
594 100
595 100
596 100
597 100
598 100
599 100
600 100
601 100
602 100
603 100
604 100
605 100
606 100
607 100
608 100
609 100
610 100
611 100
612 100
613 100
614 100
615 100
616 100
617 100
618 100
619 100
620 100
621 100
622 100
623 100
624 100
625 100
626 100
627 100
628 100
629 100
630 100
631 100
632 100
633 100
634 100
635 100
636 100
637 100
638 100
639 100
640 100
641 100
642 100
643 100
644 100
645 100
646 100
647 100
648 100
649 100
650 100
651 100
652 100
653 100
654 100
655 100
656 100
657 100
658 100
659 100
660 100
661 100
662 100
663 100
664 100
665 100
666 100
667 100
668 100
669 100
670 100
671 100
672 100
673 100
674 100
675 100
676 100
677 100
678 100
679 100
680 100
681 100
682 100
683 100
684 100
685 100
686 100
687 100
688 100
689 100
690 100
691 100
692 100
693 100
694 100
695 100
696 100
697 100
698 100
699 100
700 100
701 100
702 100
703 100
704 100
705 100
706 100
707 100
708 100
709 100
710 100
711 100
712 100
713 100
714 100
715 100
716 100
717 100
718 100
719 100
720 100
721 100
722 100
723 100
724 100
725 100
726 100
727 100
728 100
729 100
730 100
731 100
732 100
733 100
734 100
735 100
736 100
737 100
738 100
739 100
740 100
741 100
742 100
743 100
744 100
745 100
746 100
747 100
748 100
749 100
750 100
751 100
752 100
753 100
754 100
755 100
756 100
757 100
758 100
759 100
760 100
761 100
762 100
763 100
764 100
765 100
766 100
767 100
768 100
769 100
770 100
771 100
772 100
773 100
774 100
775 100
776 100
777 100
778 100
779 100
780 100
781 100
782 100
783 100
784 100
785 100
786 100
787 100
788 100
789 100
790 100
791 100
792 100
793 100
794 100
795 100
796 100
797 100
798 100
799 100
800 100
801 100
802 100
803 100
804 100
805 100
806 100
807 100
808 100
809 100
810 100
811 100
812 100
813 100
814 100
815 100
816 100
817 100
818 100
819 100
820 100
821 100
822 100
823 100
824 100
825 100
826 100
827 100
828 100
829 100
830 100
831 100
832 100
833 100
834 100
835 100
836 100
837 100
838 100
839 100
840 100
841 100
842 100
843 100
844 100
845 100
846 100
847 100
848 100
849 100
850 100
851 100
852 100
853 100
854 100
855 100
856 100
857 100
858 100
859 100
860 100
861 100
862 100
863 100
864 100
865 100
866 100
867 100
868 100
869 100
870 100
871 100
872 100
873 100
874 100
875 100
876 100
877 100
878 100
879 100
880 100
881 100
882 100
883 100
884 100
885 100
886 100
887 100
888 100
889 100
890 100
891 100
892 100
893 100
894 100
895 100
896 100
897 100
898 100
899 100
900 100
901 100
902 100
903 100
904 100
905 100
906 100
907 100
908 100
909 100
910 100
911 100
912 100
913 100
914 100
915 100
916 100
917 100
918 100
919 100
920 100
921 100
922 100
923 100
924 100
925 100
926 100
927 100
928 100
929 100
930 100
931 100
932 100
933 100
934 100
935 100
936 100
937 100
938 100
939 100
940 100
941 100
942 100
943 100
944 100
945 100
946 100
947 100
948 100
949 100
950 100
951 100
952 100
953 100
954 100
955 100
956 100
957 100
958 100
959 100
960 100
961 100
962 100
963 100
964 100
965 100
966 100
967 100
968 100
969 100
970 100
971 100
972 100
973 100
974 100
975 100
976 100
977 100
978 100
979 100
980 100
981 100
982 100
983 100
984 100
985 100
986 100
987 100
988 100
989 100
990 100
991 100
992 100
993 100
994 100
995 100
996 100
997 100
998 100
999 100
1000 100
1001 100
1002 100
1003 100
1004 100
1005 100
1006 100
1007 100
1008 100
1009 100
1010 100
1011 100
1012 100
1013 100
1014 100
1015 100
1016 100
1017 100
1018 100
1019 100
1020 100
1021 100
1022 100
1023 100
1024 100
1025 100
1026 100
1027 100
1028 100
1029 100
1030 100
1031 100
1032 100
1033 100
1034 100
1035 100
1036 100
1037 100
1038 100
1039 100
1040 100
1041 100
1042 100
1043 100
1044 100
1045 100
1046 100
1047 100
1048 100
1049 100
1050 100
1051 100
1052 100
1053 100
1054 100
1055 100
1056 100
1057 100
1058 100
1059 100
1060 100
1061 100
1062 100
1063 100
1064 100
1065 100
1066 100
1067 100
1068 100
1069 100
1070 100
1071 100
1072 100
1073 100
1074 100
1075 100
1076 100
1077 100
1078 100
1079 100
1080 100
1081 100
1082 100
1083 100
1084 100
1085 100
1086 100
1087 100
1088 100
1089 100
1090 100
1091 100
1092 100
1093 100
1094 100
1095 100
1096 100
1097 100
1098 100
1099 100
1100 100
1101 100
1102 100
1103 100
1104 100
1105 100
1106 100
1107 100
1108 100
1109 100
1110 100
1111 100
1112 100
1113 100
1114 100
1115 100
1116 100
1117 100
1118 100
1119 100
1120 100
1121 100
1122 100
1123 100
1124 100
1125 100
1126 100
1127 100
1128 100
1129 100
1130 100
1131 100
1132 100
1133 100
1134 100
1135 100
1136 100
1137 100
1138 100
1139 100
1140 100
1141 100
1142 100
1143 100
1144 100
1145 100
1146 100
1147 100
1148 100
1149 100
1150 100
1151 100
1152 100
1153 100
1154 100
1155 100
1156 100
1157 100
1158 100
1159 100
1160 100
1161 100
1162 100
1163 100
1164 100
1165 100
1166 100
1167 100
1168 100
1169 100
1170 100
1171 100
1172 100
1173 100
1174 100
1175 100
1176 100
1177 100
1178 100
1179 100
1180 100
1181 100
1182 100
1183 100
1184 100
1185 100
1186 100
1187 100
1188 100
1189 100
1190 100
1191 100
1192 100
1193 100
1194 100
1195 100
1196 100
1197 100
1198 100
1199 100
1200 100
1201 100
1202 100
1203 100
1204 100
1205 100
1206 100
1207 100
1208 100
1209 100
1210 100
1211 100
1212 100
1213 100
1214 100
1215 100
1216 100
1217 100
1218 100
1219 100
1220 100
1221 100
1222 100
1223 100
1224 100
1225 100
1226 100
1227 100
1228 100
1229 100
1230 100
1231 100
1232 100
1233 100
1234 100
1235 100
1236 100
1237 100
1238 100
1239 100
1240 100
1241 100
1242 100
1243 100
1244 100
1245 100
1246 100
1247 100
1248 100
1249 100
1250 100
1251 100
1252 100
1253 100
1254 100
1255 100
1256 100
1257 100
1258 100
1259 100
1260 100
1261 100
1262 100
1263 100
1264 100
1265 100
1266 100
1267 100
1268 100
1269 100
1270 100
1271 100
1272 100
1273 100
1274 100
1275 100
1276 100
1277 100
1278 100
1279 100
1280 100
1281 100
1282 100
1283 100
1284 100
1285 100
1286 100
1287 100
1288 100
1289 100
1290 100
1291 100
1292 100
1293 100
1294 100
1295 100
1296 100
1297 100
1298 100
1299 100
1300 100
1301 100
1302 100
1303 100
1304 100
1305 100
1306 100
1307 100
1308 10
```