



- (51) **International Patent Classification:**
C12Q 1/6869 (2018.01)
- (21) **International Application Number:**
PCT/EP2021/086349
- (22) **International Filing Date:**
16 December 2021 (16.12.2021)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
63/127,043 17 December 2020 (17.12.2020) US
- (71) **Applicant: ILLUMINA CAMBRIDGE LIMITED**
[GB/GB]; 19 Granta Park, Great Abington, United Kingdom, Cambridge Cambridgeshire CB21 6DF (GB).
- (72) **Inventors: LIU, Xiaohai;** c/o Illumina Cambridge Limited, 19 Granta Park, Great Abington, United Kingdom, Cambridge Cambridgeshire CB21 6DF (GB). **WU, Xiaolin;** c/o Illumina Cambridge Limited, 19 Granta Park, Great Abington, United Kingdom, Cambridge Cambridgeshire CB21

6DF (GB). **MCCAULEY, Patrick;** c/o Illumina Cambridge Limited, 19 Granta Park, Great Abington, United Kingdom, Cambridge Cambridgeshire CB21 6DF (GB). **ROMANOV, Nikolai Nikolaevich;** c/o Illumina Cambridge Limited, 19 Granta Park, Great Abington, United Kingdom, Cambridge Cambridgeshire CB21 6DF (GB). **CRESSINA, Elena;** c/o Illumina Cambridge Limited, 19 Granta Park, Great Abington, United Kingdom, Cambridge Cambridgeshire CB21 6DF (GB). **FRANCAIS, Antoine;** c/o Illumina Cambridge Limited, 19 Granta Park, Great Abington, United Kingdom, Cambridge Cambridgeshire CB21 6DF (GB).

(74) **Agent: MURGITROYD & COMPANY;** Murgitroyd House, 165-169 Scotland Street, Glasgow Strathclyde G5 8PL (GB).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN,

(54) **Title:** METHODS, SYSTEMS AND COMPOSITIONS FOR NUCLEIC ACID SEQUENCING

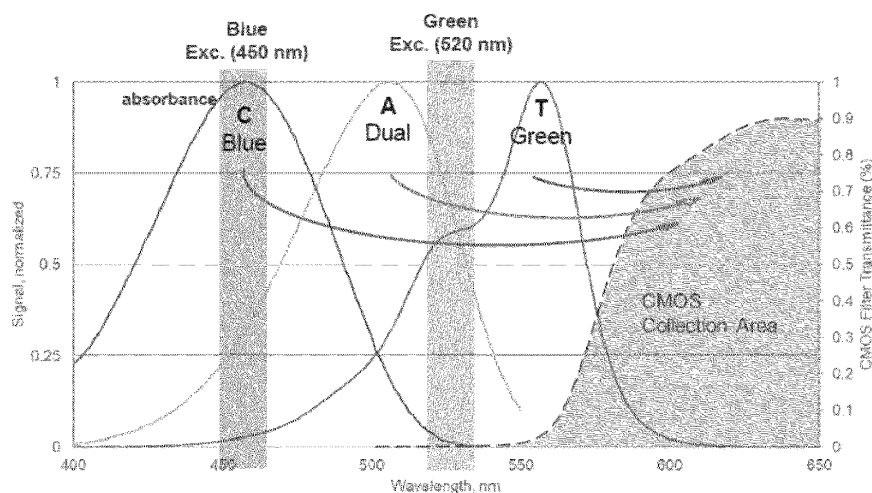


FIG. 2

(57) **Abstract:** The present disclosure relates to methods, systems, kits and compositions for nucleic acid sequencing applications. In particular, the method utilizes two imaging events with different excitation wavelengths and a single emission channel to collect the fluorescent signal patterns of different types of nucleotide conjugates to determine the identity of the incorporated nucleotide conjugates. The method described herein does not require a chemical treatment of the nucleotide conjugates in the incorporation mixture between the two imaging events.

[Continued on next page]

KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*