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Original Article

Quantitative analysis of operators' flow line in the cell culture for controlled manual operation

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ABSTRACT

Introduction: Although cell culture has been widely used in the life sciences, there are still many aspects of this technique that are unclear. In this study, we have focused on the manual operations in the cell culture process and try to analyze the operators' flow line.
Methods: During a course of approximately 6 years, we obtained the operators' flow line data from two places (three layouts) and 38 operators (93 subcultures) using two network cameras and a motion detection software (Vitracom SiteView).
Results: Our investigation succeeded in quantifying the flow line of the subculture process and analyzed the time taken to carry out the process, to travel around the workplace. For the subculture process, the total time of the process being rerated the time of the operation in the place where the main operation is performed; the total distance of travel and the counts of travel not being related to the total time of the process. Based on these results, we propose a new way of evaluating the efficiency of cell culture process in terms of time and traveling. We believe that the results of this study can guide cell culture operators in handling cells more efficiently in cell manufacturing processes.
Conclusions: The flow line analysis method suggested by us can record the operators involved and improve the efficiency and consistency of the process; it can, therefore, be introduced in cell manufacturing processes. In addition, this method only requires network cameras and motion detection software, which are inexpensive and can be set up easily.

1. Introduction

Since the establishment of the cell culture as an important molecular biology tool in 1907 by Harrison, animal and human cell culture have been widely used in life science research [1]. Cell culture is increasingly being applied to a wide variety of fields such as cancers, stem cells, drug discovery, biomaterials, and regenerative medicine.

In the pharmaceutical field, cell-based assays are routinely used to measure cell proliferation, toxicity, production of cellular markers, motility, activation of specific signaling pathways, and changes in morphology for the early phase of drug-discovery process [2]. To construct good models, different cell culture techniques such as 3D culture [3], organ-on-chip platform [4], and induced pluripotent stem cells (iPSCs) derived *in vitro* disease modeling are developing [5]. In recent years, phenotypic drug discovery approaches have contributed strongly to the discovery of the first-in-class drugs [6,7]. In addition to drug discovery, cell culture is also used to manufacture new types of drugs, such as antibody drugs [8].

In the field of tissue engineering and regenerative medicine, clinical studies associated with bioprinting [9,10], cell sheets [11], chimeric antigen receptor (CAR) T cells [12], mesenchymal stem

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cells (MSCs) [13,14], embryonic stem cells (ESCs) and iPSCs [15,16] are conducted. In order to develop regenerative medical products unique in structural and functional complexity compared with traditional chemical drugs, legislations were enacted in various countries [17,18]. In Japan, two new laws were implemented to promote regenerative medicine on November 25, 2014 [19]. The field of cell culture is not only undergoing technological advancements (improved devices), but also advancements in facilities (cell processing center) and training of cell culture operators [20,21].

However, current standardization studies in cell processing are extremely few, only studies focusing on the safety of cell products preventing an intrinsic contamination from microorganisms exist [22]. In addition, since the process of cell culture is long and complicated, it is very difficult to understand the factors affecting cell properties (proliferation potential, differentiation potential, and therapeutic effect). In order to manage the manufacturing process of cells that are difficult to confirm or understand, the concept of quality by design (QbD), first introduced in the biopharmaceutical field, is important [23,24]. QbD is a systematic approach towards process and product management based on scientific knowledge and risk assessment. In short, consistency, efficiency, feasibility, and cost are important factors involved in cell product manufacturing. In order to thoroughly quantify, describe, record, and analyze the cell culture process, it is important to firstly understand the situation well [25]. In order to quantify changes in the cell culture process, describing each operation in detail is of utmost importance. In addition, if the correlation between the quantitative and qualitative analysis of each operation is deduced, there is a high possibility of obtaining information that can enhance the process. The authors have shown that analysis of cell morphology image information during cell culture is an effective method to determine the progress of the cell culture process [26–29]. However, a few studies are quantifying and analyzing the preliminary stage of image evaluation which is said to vary largely among researchers. Currently, many cell culture operations are automated, but several operations still require manual operation and need quantification. Thus, it is extremely important to carry out the quantitative analysis and to know the influence on the cell manufacturing process about the manual operation evaluated even now with unclear criteria “good/bad” operation. In this research, we tried to quantify and analyze operators' flow line of subculture process, which is the most basic procedure in cell culture (Fig. 1). Through about 6 years, we obtained the operators' flow line data of subculture process in 2 places (3 different layouts) and 38 operators (total 93 subculture processing data) to use only 2 network cameras and 1 motion detection software (Vitracom SiteView). From obtained data, we visualized the operators’ flow line as timestamp graph, halt time of each area, spent time of each operation, distance and counts of travel, and correlation with total time or subculture process (what is the relationship between operators who operate fast and slow), and discuss the importance of quantitative understanding of cell culture process from the analyzed data.

2. Methods

2.1. Video-based analysis

To obtain operator's flow line data of subculture process, only 2 tools were used throughout this study. These are the network camera and the motion detection software. Two network cameras (camera 1: M1114 [Axis Communications AB, Lund, Sweden], camera 2: M1011-W [Axis Communications AB]) were installed in our laboratory to cover the operation area where the operator may stop or pass through (measurement area). For each layout, the positions of the network cameras and each measurement area have been shown in Fig. 2A–C. Vitracom SiteView (version 3.7.68, Vitracom AG, Karlsruhe, Germany) was used to detect the locations of moving objects from individual cameras. This software has otherwise been exploited to measure the number of visitors in the retail stores, measuring the flow of customers in the store, operation of workers in the manufacturing industry, and measurement of working time as a marketing and work analysis tool [30,31]. The method of software configuration for data measurement is to frame the area to be measured. During measurement, the status of the blue frame is not detected, and the status of the red frame is motion detection (Fig. 1). And the detected information is recorded along with the time axis. From the detected data, position information was extracted every 10 s by a script created by Ruby (version 2.2.0) (https://www.ruby-lang.org/ja/downloads/).

2.2. Distance calculation and data analyses

The distance between each measurement area was measured with the 3D CAD software using Sweet Home 3D (version 6.0, eTeks) (http://www.sweethome3d.com/ja/). All the data analyses were carried out by Microsoft Excel 2016.

2.3. Cells and cell culture

Normal human dermal fibroblasts (KF-4109, KURABO, Osaka, Japan) and HT-1080 (ATCC® CCL-121™, ATCC, VA, U.S.A.) were maintained in Dulbecco's modified Eagle's medium (DMEM) (044-29765, Wako Pure Chemical Industries, Osaka, Japan) containing 10% fetal bovine serum (Nichirei Biosciences, Tokyo, Japan) and 1% penicillin-streptomycin (168-23191, Wako Pure Chemical Industries). The cells were maintained at 37 °C with 5% CO_2 and were used within 4–6 passages.

2.4. Operator and subculture protocol

The operator who performed the subculture operation received experimental guidance in our laboratory and practiced at least once. In order to perform consistent subculture process, the following 8 rules were established: 1. The operator should stay in the room once his/her measurement starts. 2. He/she should start with the measurer's signal and end with the signal of the operator. 3. Cleaning up should be included as a part of the process. 4. Warming duration of trypsin and the culture media should be 15 min (to be measured with a timer). 5. The trypsin treatment time should be 3 min (to be measured with a timer). 6. The centrifugation time should be 5 min. 7. Subculture should be performed from two 80% confluent 10 cm dishes to four 10 cm dishes. 8. After cell counting, 5×10^5 cells/dish should be calculated and seeded. The schematic diagrams are shown in Fig. 2D and E. There are two operation types of subculture process in our laboratory. There are five operations in either of the two operation types (Op1: preparation, Op2: cell collection, Op3: cell count, Op4: seeding and Op5: clean up). In operation of preparation, the operator prepares the necessary materials for subculture processes during 15 min of warming the medium, trypsin and PBS. In operation of cell collection, the operator brings the cell-containing dish placed in the incubator and the medium, trypsin and PBS contained in the water bath to the clean bench. Washing with PBS and injection of trypsin are performed in a clean bench, and then the dishes are placed in an incubator for 3 min and cells are trypsinized. After 3 min, transfer the dishes from the incubator to a clean bench (after

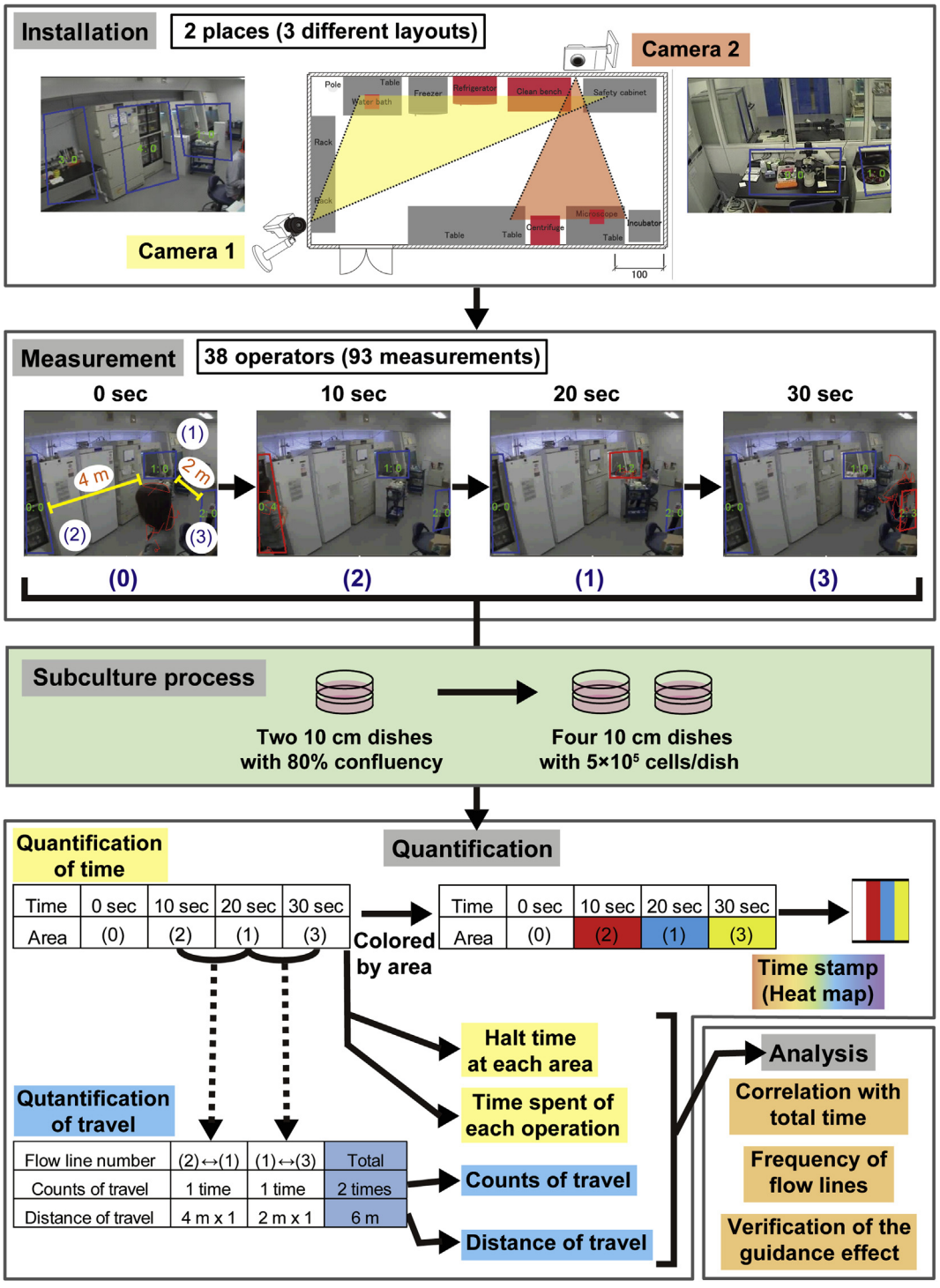

Fig. 1. Schematic design of the study to quantify the subculture operation. Installation: Two network cameras were used in 2 places (3 different layouts). Measurement: Motion detection software (Vitracom SiteView) was used for 38 operators (total 93 subculture operation data). The picture is a simple example of data acquisition. The measurement area in the figure shows (1) is clean bench, (2) is refrigerator and (3) is microscope. The distance between (1) and (2) is 4 m, and the distance between (1) and (3) is 2 m. At 0 s, no area is recognized, thus it is labeled (0). At 10 s, because it recognizes the area of the refrigerator, it is labeled (2). Subculture operation: Cells were subcultured from two 10 cm dishes with 80% confluency to four 10 cm dishes with 5×10^5 cells/dish. Quantification: Two types of numerical information were extracted for quantification. One is "Quantification of time" and the other is "Quantification of travel". Furthermore, there are "Halt time at each area" and "Time spent of each operation" in quantification of time, and "Counts of travel" and "Distance of travel" in quantification of travel. In addition, by coloring each area information, a time stamp (heat map) graph is generated. Analysis: Three analyzes: "Correlation with total time", "Frequency of flow lines" and "Verification of the guidance effect" were performed using the four quantification information.

microscopic check), collect the cells in centrifuge tubes and centrifuge for 5 min. In operation of cell count, the operator brings the sampled cell suspension into a microscope area during centrifugation (Type A) or after centrifugation (Type B), counts cells using a hemocytometer, and calculates cell concentration. In operation of seeding, the operator brings the cell suspension after centrifugation to a clean bench and adjusts it to the required concentration. The prepared cell suspension is

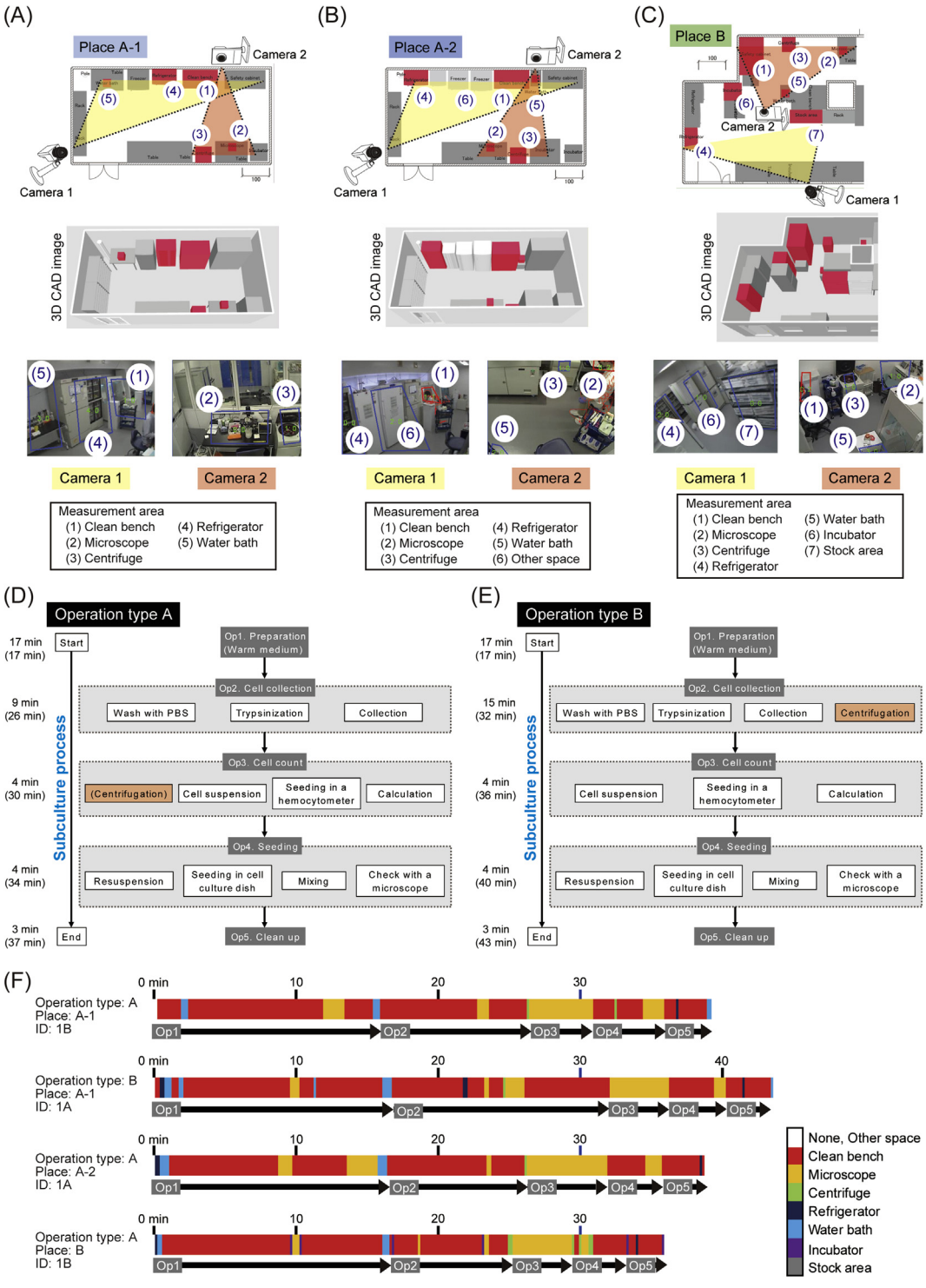

Fig. 2. Methodology employed in this study. The layout, the installation position of the network cameras and the images of measuring area at 2 places (3 different layouts) are shown. (A) At place A-1, (B) at place A-2 and (C) at place B. The flow chart of subculture process; the displayed time is the ideal time. (D) Operation type A and (E) Operation type B. (F) Timestamp graph of the operator who performed the ideal subculture process. The ID represents one operator and each color represents each measured area where the operator stayed. For example, red colored band represents the clean bench where the operator stayed. Op means operation. Op1: preparation, Op2: cell collection, Op3: cell count, Op4: seeding and Op5: clean up.

seeded in dishes and (after microscopic check) transferred to the incubator. In operation of clean up, the operator discards the disposable product and returns the rest to its original position. The visualized figures of ideal subculture process were shown in Fig. 2F. In either of the operation type and at either place, the subculture process completes in around 40 min ideally.

3. Results

3.1. Visualization of operators flow line by timestamp graph (heat map)

Fig. 3 shows the timestamp graph of operators' flow line measurements at each place. In order to eliminate the operator's

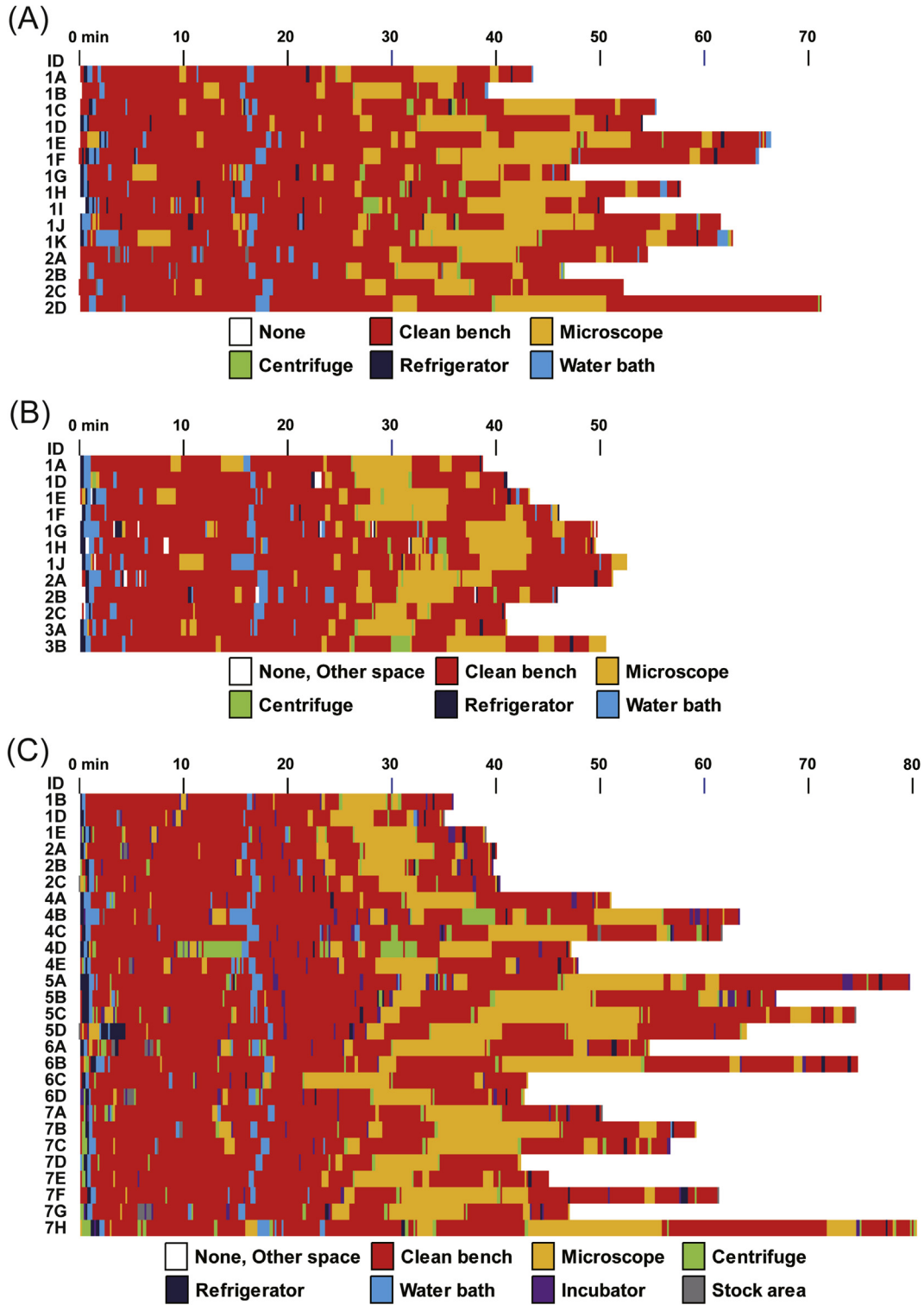

Fig. 3. The results of the timestamp graph of the operator's flow line of the subculture process. (A) At place A-1, (B) At place A-2 and (C) At place B.

experience from repeated work, the graph shows the result of only the first measurement at each place. These results indicated that even in the simplest subculture process of cell culture, the patterns of time and travel were different for each person. Specifically, the fastest person (operator ID: 1D) finishes work in 35.2 min, whereas the slowest person (operator ID: 7H) takes 80.7 min, and the time difference between individuals varies up to 2.3-fold between individuals. In addition, two mistakes were detected in the process (Fig. S1A and S1B) and the different types of operation were detected (Fig. S1C) and one characteristic movement (The operator (ID: 4D) stood in front of the centrifuge for the waiting time.) were detected (Fig. 3) during the measurement period could also be visualized. From this result, it was found that it is possible to understand and detect time and travel, which are not otherwise noticed, by measuring and visualizing the subculture process.

3.2. Time analysis of operation area in subculture process (halt time at each operation area)

In order to investigate the cause of the difference in subculture process time in detail, operator's flow line results were classified based on total time of the process using population mean (50.1 min, N = 93) and standard deviation (10.0 min) as reference values. Using this value, the category was determined so that the average value of class A would be approximately 50 min, class S would be approximately 40 min (minus 1 SD (10 min)), class B would be approximately 60 min (plus 1 SD (10 min)) and class C would be approximately 70 min (plus 2 SD (20 min)) (the class S < 45 min, 45 min ≤ the class A < 55 min, 55 min ≤ the class B < 65 min, 65 min ≤ the class C). A summary of the results of all the 93 measurements for each class is shown in Fig. S2A (Detailed data are shown in Tables S1, S2, and S3). The average value of each class was 41.0 min for S (N = 34), 49.6 min for A (N = 36), 60.0 min for B (N = 15), and 72.5 min for C (N = 8). The result of the difference in total time with the class S is shown in Fig. S2B. The comparison of S and C showed a maximum of 31.5 min difference in this classification.

Next, by comparing each class, we examined the difference in the time of the place of staying, with fast operation and slow operation. Fig. 4A shows the results of the halt time in each operation area, and Fig. S2C shows the difference with respect to class S. These results suggest that the operation area where the total time difference is mainly generated is at the clean bench and the microscope. Furthermore, the correlation between the total operation time and the halt time at each place is shown in Fig. 4B. The correlation coefficient with the clean bench is 0.94, and with the microscope is 0.84. These values are very high, and the correlation coefficient with combined time (clean bench + microscope) is up to 0.98. This clearly indicates that the crucial factors related to the total time difference are the clean bench and the microscope, which also shows that these are the main operation areas of the subculture process. These results indicate that it is possible to determine the main operation during the cell culture process by performing flow line analysis.

3.3. Time analysis of the operation in subculture process (time spent per operation)

In order to determine the operating time mainly spent in the subculture process, the time consumed for each of the five operations involved in the same (preparation, cell collection, cell counting, seeding, clean up) was examined per class. The result is shown in Fig. 5A (detailed data are shown Table S4), and the difference with respect to class S is shown in Fig. S3. Slow operators were found to spend more time on cell collection, cell seeding, and cell counting processes. The maximum time difference between slow and fast operators was 11.6 min during the cell collection step, 9.1 min in the cell seeding and 6.4 min in the cell count process. Furthermore, the association between the total time and the time spent of operation is shown in Fig. 5B. From these results, the correlation coefficient for cell collection was 0.83, for cell seeding was 0.78 and for cell count was 0.75, indicating a higher correlation than other processes of the operation. These values are very high, and the correlation coefficient with combined time (cell collection + cell count + seeding) is up to 0.98. In other words, flow line analysis helped us understand that there is some operation that influence the total time of the subculture process more than others.

3.4. Analysis of operator's total distance and total counts of travel (regarding operator's travel)

In the previous sections, we discussed the time spent (halt time in area and time spent of operation) of the operators, here, we focus on the travel of the operator. From the viewpoint of operation efficiency, it is not only important to reduce the time spent itself, but equally important to reduce the wasteful travel (distance or counts) too. In order to calculate the total distance of travel for each operator, the distance between the operation areas at each place was measured (Fig. S4). Using the results of Fig. S4, the total distance and total counts in travel for each operator were calculated (detailed data are shown Table S5). Regarding the distance of travel, the minimum was 32 m and the maximum was 141.9 m, which was about 4.4-fold the difference. With regard to the counts of travel, the minimum was 14 times and the maximum was 55 times, about 3.9-fold higher. In order to arrange the results, classification was performed for the counts of travel (class S' < 25 times, 25 times ≤ the class A' < 35 times, 35 times ≤ the class B' < 45 times, 45 times ≤ the class C'). Fig. 6A and Fig. S5A show the results of the total distance of travel sorted by classes, total counts of travel, and the correlation between distance and counts of travel. The average values of distance of travel in each class were: 46.4 m for S' (N = 17), 65.3 m for A' (N = 26), 86.3 m for B' (N = 33), and 104.9 m for C' (N = 17). The average value of the counts of travel in each class was 20.6 times for S' (N = 17), 30.3 times for A' (N = 26), 38.1 times for B' (N = 33), and 47.4 times for C' (N = 17). The correlation coefficient between the distance and the counts of travel was calculated to be as high as 0.94, and it is suggested that an operator with a higher number of travel tends to travel a longer distance.

Next, the relationship between the total time and distance of travel (Fig. 6A, Fig. S5B), and counts of travel (Fig. 6A, Fig. S5B) was investigated. The analysis suggested that neither the distance nor the counts of travel showed a difference between classes (total time), and the correlation coefficient was found to be very small (0.31: vs distance of travel, 0.33: vs counts of travel). From these results, it was found that the total time of the operator and the travel (total distance or count) of the operator are independent in this subculture process.

Furthermore, in order to investigate the efficiency of operator movement in detail, we focused on the frequency of flow lines (lines connecting two specific operation areas). Fig. 6B shows the counts of travel on each flow lines (It shows which flow line the operator frequently uses.) at each place, and Fig. S5C shows the distance of travel. Although the measurement place and the measured operator are different, it was found that the frequency of the flow lines involving the clean bench ((1)↔(1), (1)↔(2), (1)↔(3), (1)↔(4), and (1)↔(5)), which is the main operation area, is higher than the other flow lines (colored with red). Besides, the flow lines (4)↔(5) and (2)↔(3) are also relatively frequent (colored with green). This indicates that the operator is

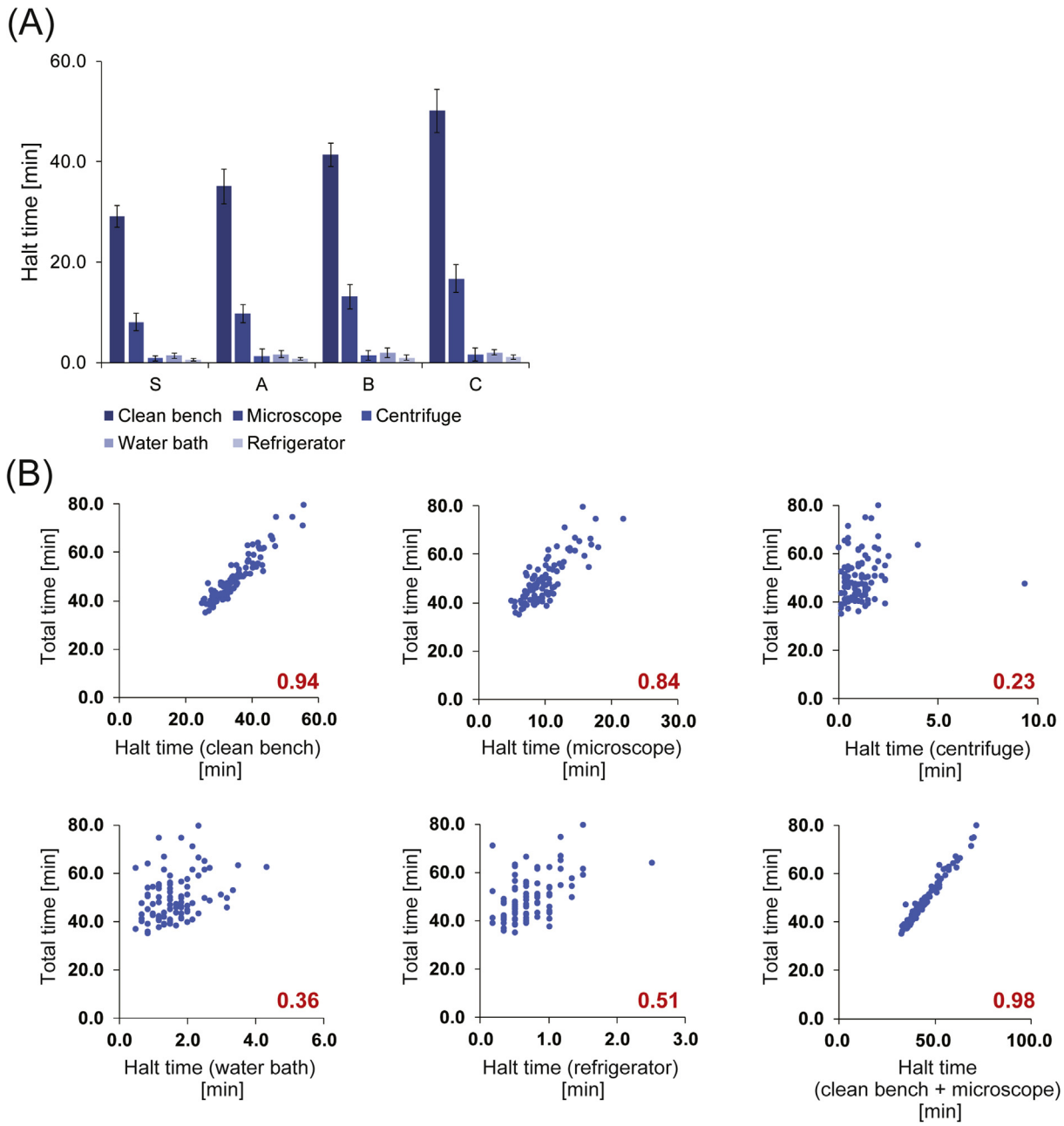

Fig. 4. The results of time analysis of the operation area in subculture process (halt time at each operation area). (A) Halt time at each place and each class. (B) Correlation diagram between total time and halt time of each area. Red numbers indicate the correlation coefficients.

continuously using the flow line of refrigerator and water bath ((4)↔(5)), the flow line of the microscope and the centrifuge ((2)↔(3)). Fig. 6C and D shows the difference in distance of travel due to differences in arrangement of equipment related to operation. For example, in case of flow line number (1)↔(5) (between clean bench and water bath), the difference in flow line frequency at place A-1 and A-2 of class B′ is 9.5 times (place A-1) and 9.7 times (place A-2). However, since the distance of the flow line number (1)↔(5) decreased from 3.3 m (place A-1) to 0.9 m (place A-2), the difference in the distance of travel with class B′ decreased from 31.4 m (place A-1), to 8.7 m (place A-2) (Fig. 6C). On the other hand, for flow line number (1)↔(4) (between clean bench and refrigerator), the difference in flow line frequency at place A-1 and B of class B′ is 5.2 times (place A-1) and 3.2 times (place B) However, since the distance of the flow line number (1)↔(4) increases from 1.2 m (place A-1) to 4.8 m (place B), the difference in the distance of travel with class B' increased from 6.2 m (place A-1), to 15.5 m (place B) (Fig. 6D). Thus, it is suggested that even if the number of movement count does not change, there is a difference in the final distance of travel depending on the distance of the flow lines (the distance of each equipment related to operation). In other words, the layout of positioning of different objects could influence the flow line efficiency in cell culture process.

3.5. *Verification of the guidance effect using flow line analysis*

We speculated that one of the applications employed by flow line analysis is to improve the operation efficiency of operators.

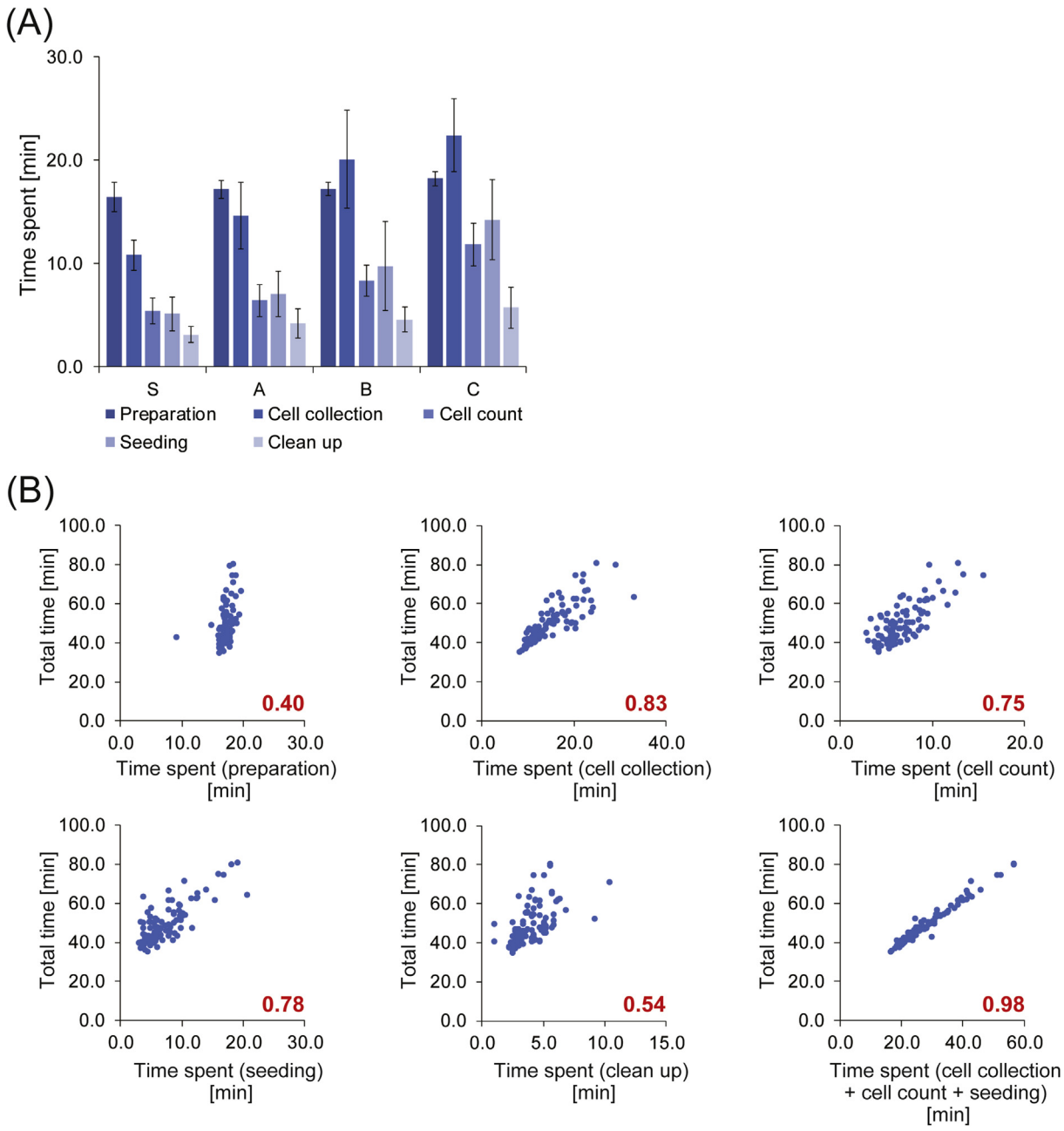

Fig. 5. The results of the time analysis of the operation in subculture process (Time spent per operation). (A) Time spent of each operation and each class. (B) Correlation diagram between total time and time spent of the operation. Red numbers indicate the correlation coefficients.

This improved efficiency will lead to consistency as well as competence. In order to verify the effect of the flow line analysis, an experiment was conducted to improve operation efficiency of operator using the result of the flow line analysis. For the instructing method, the flow line analyses results (mainly time-stamp graphs) were used. For example, compared to operators of class S, it would be possible to clearly state the time taking operation(s). In order to carry out this verification, a repeat experiment was conducted in which the same operator performs the subculture process thrice. In order to eliminate habituation by prior experience/adaptation, these three iterative experiments were performed after approximately a week. Furthermore, in order to compare with the effect of habituation due to repeated experiences, an operator without guidance was also prepared and compared. The results of the time spent to perform each process are shown in Fig. 7A and Fig. S7A, and the results of the total distance and the total counts of travel are shown in Fig. 7B. Fig. 7A shows that the total time decreases by repeating the operation regardless of the instruction given. However, Fig. 7B shows that total distance and total counts of travel related to the flow line efficiency hardly changed regardless of guidance and/or repetition experiments. Besides, in order to explore which operation is influenced by guidance, the change in the time of each operation was analyzed, as shown in Fig. 7C (without guidance) and Fig. 7D (with guidance). Firstly, compared with the un-guided operators, the total time of the guided operators sharply decreased in the second experiment. Moreover, as a result of detailed verification of each operation, the operations contributing to the sharp decrease

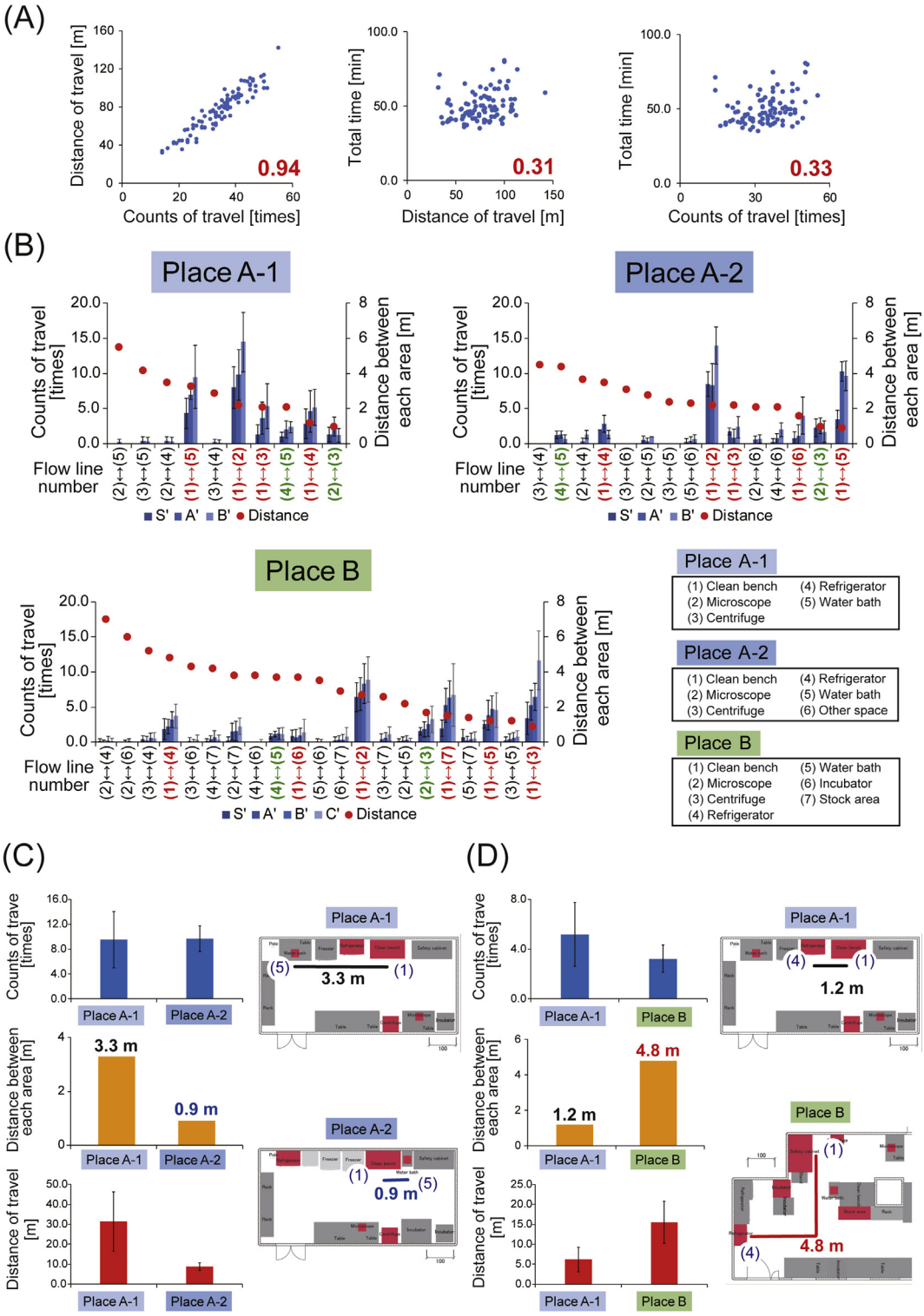

Fig. 6. Analysis of operator's total distance and total counts of travel (Regarding operator's travel). (A) The correlation between distance and counts of travel, the total time and total distance of travel and the total time and the total counts of travel. (B) The results of the counts of travel on each flow line at each place. The data is arranged in order of long distance of flow line. Red dot means the distance between two specific operation areas. The results of the difference in distance of travel due to differences in arrangement of equipment related to operation. (C) The difference in flow line frequency at place A-1 and A-2 of class B′ in case of flow line number (1)↔(5). (D) The difference in flow line frequency at place A-1 and B of class B′ in case of flow line number (1)↔(4).

in total time are cell collection, cell counting, and seeding. In particular, it is suggested that the time reduction rate is high for the cell collection operation, which is greatly affected by guidance mediated operation improvement. Moreover, if three repeat experiments were performed, the total time settled roughly at the same time, i.e. 43.7 min for the operators without guidance and 45.4 min for the operators with guidance. These results suggest a probable effect of guidance on flow line analysis, and it is

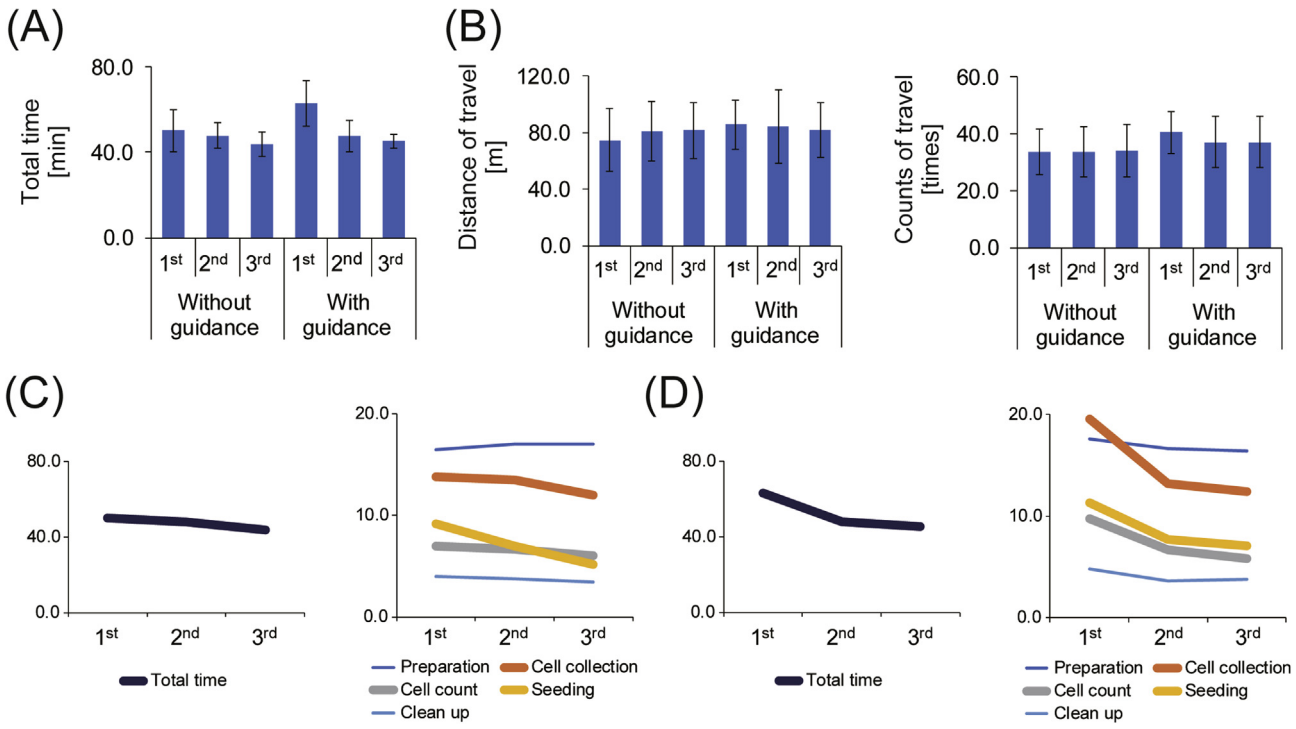

Fig. 7. Verification of the guidance effect using flow line analysis. (A) The total time and time spent on each operation with/without guidance in triplicate experiments. (B) The distance and counts of travel with/without guidance in triplicate set of experiments. Decrease in the total operation time and time spent on each operation by repeated experiments without (C) or with guidance (D).

considered that redoing the measurements paves way for efficiency and consistency of the cell culture process.

4. Discussion

Although cell culture is a necessary technology in life sciences, the quality of the cells being used can only be determined at the end point of culturing, and the criteria for a “good” or “bad” cell culture process are not well defined. Therefore, we focused on the flow line of operator in cell culture process and tried to quantify the operation of the subculture process. In this research, we quantified and analyzed data from 93 operators’ subculture flow line (a total of 38 operators) in a span of 6 years at 2 places (3 layouts). The analyses were done using only two network cameras and one motion detection software (Vitracom SiteView) (Fig. 1).

By visualizing the subculture process of the operators, it was clearly possible to exhibit the difference between the fast and slow operators (Fig. 3). Furthermore, it was also possible to detect a mistake and a difference of operation method (Fig. S1). Moreover, even if the location of the operation place changes, these quantification procedures can be carried out simply by changing the position of the camera, and accordingly updating the area to be measured in the software. It is thus justifiable to say that flow line analyses in the cell culture process can be applied to process management in the cell processing center as it facilitates the recording of the operator process and detecting any deviation from standard protocols.

In addition, we analyzed the acquired data elaborately from the viewpoint of the halt time at the operation area, and brought out the operational differences between the fast and slow operators. At that time, in order to discover the difference between the fast and slow operators, the data of flow line analyses were classified into four class using the total time of subculture process (Fig. S2A). Consequently, we found a difference between fast and slow operators in the halt times of the clean bench and the microscope areas, where the main operation was performed (Fig. S2C). Also, and the correlation with the total operation time was also very high (Fig. 4B). This showed that the difference in the total time of the subculture process arises due to the time spent at the clean bench and the microscope area. In other words, raising the efficiency of the operation done at the clean bench and the microscope area may possibly lead to an improvement in the total operational potential.

Next, we investigated the operation contributing to the difference between the fast and slow operators. It was seen that the time spent during cell collection, seeding, and cell counting process remarkably created this difference (Fig. S3); and these three processes were also highly correlated with the total operational duration (Fig. 5B). This result seems to be all the more reasonable because cell collection and seeding are mainly performed in the clean bench area and cell count is performed at the microscope. Furthermore, in the cell collection operation, even the class A operators had a difference of 3.8 min compared with the class S operators, which means that it is difficult to reduce the time of this operation. In this specific case, therefore, an improvement plan that mechanizes only this operation can be considered in order to increase the possibility of stable, independent of manual operation.

For the operational efficiency, we analyzed the flow line systematically at each place from the perspective of shortening the total time and preventing unnecessary travel. For this purpose, the total distance and the total counts of travel of the operators were calculated, and fresh class was made according to the total counts of travel (Fig. 6A, Fig. S5A). The results proved that even the time spent of subculture process is same, there are operators who move frequently and operators who do not move much. It was also found that the total distance and the total counts of travel were hardly correlated with the total time of the process, and hold no relevance in the operation speed and travel during the subculture process. In short, we suggest that even if the total distance and counts of travel

are higher, the total duration may be shortened when the main operation time is reduced. Therefore, we propose a new operation efficiency class system (S″, A″, B″, and C″) which considers the class of the total time of the process (S, A, B, and C) and the class of the total count of travel (S′, A′, B′ and C′) independently. The categorization is simple. For example, an operator classed both S and S' is set as S''. When either one is S or S ', it is classified as the lowest class. For example, an operator classed S and C″ is set as C" (Fig. S6, detailed data are shown Table S6).

From another perspective, it is important to reduce the total distance and counts of travel, since this would help in reducing operator's labor and the particle count in the cell processing center, in turn leading to a reduced risk of human error and contamination. It is for this reason that the flow line known to move more shortens the flow line distance and vice versa. To sum it up, there is a necessity to consider arrangement and layout while analyses. The arrangement of the apparatus is close by for the frequently moving flow line, and far for the flow line which is known not to move much.

Finally, we investigated how operator efficiency was influenced by the use of flow line analysis (Fig. 7). The results showed that the total time of guided operators sharply decreased in the second experiment compared to the unguided operators using the result of the flow line measurements. For reference, Figs. S7B and S7C show the change in the total operation time and the time spent on each of the processes for 5 operators over 3 years. The total time decreased from 56.5 min in the first year to 44.7 min and 39.0 min in the second and third years, respectively. It was thought that the effectiveness of guidance was significant even if the total time was shortened within a span of just 3 weeks. However, while the guidance effect could shorten the total time (Fig. 7A), it could not shorten the total distance and counts of travel (Fig. 7B). Thus, another approach is required to reduce the total distance and counts. For example, it is necessary to designate not only the operation time but also the place to go, the tasks/procedures to do, the tools to be prepared, etc. in a more detailed and apparent protocol.

Our study suggests that the flow analysis performed by us has the potential to be introduced into the cell manufacturing process because the analysis could record the operators, along with improving the efficiency and consistency of the operation. In addition, this method uses only inexpensive network cameras and motion detection software, and can be installed easily. So it is considered to be cost effective and feasible in terms of cell manufacturing. Although this analytical evaluation can be used independently, we speculate that it can also be used as a part of the lengthy cell production process. Here we propose a methodology to link all the information which participates in cell production, such as information of cell source, cell culture medium and vessel, cell morphology, cell proliferation and differentiation, and so on. In other words, we believe that this research is the first step to introduce the idea of the so-called IoE (Internet of Everything) into the cell manufacturing process [32].

5. Conclusions

In this study, to quantify the undefined steps of cell culture, quantification of subculture operation was performed using only network cameras and motion recognition software. We succeeded in quantifying the subculture operation irrespective of its location and also believe that a stable measurement system can be established. Also, in the time analysis of the operation area, it was found that the halt time at the clean bench and the microscope areas, where the operation is mainly performed, directly influences the total time of the process. Furthermore, in the time analysis of each operation, it was found that the operations of cell collection, seeding, and cell counting highly affected the total time. Additionally, no correlation was observed between the total time and total distance, and counts in travel in our analyses. All these results encouraged us to propose a new class that evaluates the efficiency of cell culture operation in terms of speed and travel. Furthermore, we also suggest that the flow line analysis of this study can be effectively used for guiding cell culture operators in general. In our opinion, the evaluation method developed in this research will be helpful for assessing cell manufacturing processes in the future.

Conflicts of interest

None.

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Appendix A. Supplementary data

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