A Modified Multiple Alignment Fast Fourier Transform with Higher Efficiency

Weihua Zheng, Kenli Li, Keqin Li, and Hing Cheung So

Abstract—Multiple sequence alignment (MSA) is the most common task in bioinformatics. Multiple alignment fast Fourier transform (MAFFT) is the fastest MSA program among those the accuracy of the resulting alignments can be comparable with the most accurate MSA programs. In this paper, we modify the correlation computation scheme of the MAFFT for further efficiency improvement in three aspects. First, novel complex number based amino acid and nucleotide expressions are utilized in the modified correlation. Second, linear convolution with a limitation is proposed for computing the correlation of amino acid and nucleotide sequences. Third, we devise a fast Fourier transform (FFT) algorithm for computing linear convolution. The FFT algorithm is based on conjugate pair split-radix FFT and does not require the permutation of order, and it is new as only real parts of the final outputs are required. Simulation results show that the speed of the modified scheme is 107.58 to 365.74 percent faster than that of the original MAFFT for one execution of the function Falign() of MAFFT, indicating its faster realization.

Index Terms—Convolution, fast Fourier transform (FFT), MAFFT, multiple sequence alignment (MSA)

1 Introduction

Multiple sequence alignment (MSA) refers to identifying the biological similarity between three or more bioinformatic sequences. It is a basic tool in various aspects of molecular biological analysis ranging from detecting key functional residues, phylogenetic inference, structure prediction of noncoding ribonucleic acids and proteins, to inferring the evolutionary history of protein families. In the process of MSA, in order to maximize the number of similar amino acids or nucleotides, gap symbols " $-$ " are inserted in sequences. That is, we transform k ($k \geq 2$) sequences $\{s_1, s_2, \ldots, s_k\}$ into $\{s'_1, s'_2, \ldots, s'_k\}$, where each of the former, s_i, $i = 1, 2, \ldots, k$, can be expressed as a string of n amino-acid or nucleotide symbols, and s'_i is elongated version of s_i with gaps " $-$ " inserted to maximize the similar regions of the sequences. As a simple illustration, the resultant s'_1 and s'_2 in the MSA with two protein sequences s_1: $TGCAATGACC$ and s_2: $TGAATTGGTG$ are

$$\begin{array}{cccccccccc} T & G & C & A & A & T & - & G & A & C & C \\ T & G & - & A & A & T & T & G & G & T & G. \end{array}$$

When gaps are inserted into sequences, the alignment score will be reduced by the weight costs which depend on the gap locations [1].

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When k is not fixed, MSA is an NP-complete problem [2]. According to Hosni et al. [3], there are three main MSA approaches, namely, divide and conquer [4], [5], iterative method [6], [7], [8], [9], and progressive method [3], [10], [11], [12], [13]. The progressive approach is the most used and the most efficient one. It operates in three steps: (i) To determine the sequences that are the first to be aligned, the distance between every pair of sequences is computed and is stored in a symmetric diagonal matrix, called distance matrix. (ii) By using the distance matrix and constructing a guide tree, the branching order of the sequences is defined. (iii) The sequences are aligned through the guide tree. In order to enhance the multiple alignment scores, a refinement step can also be applied with the use of MUSCLE [14], MAFFT [15], and PAAP [16].

Needleman and Wunsch [17] have presented a dynamic programming (DP) for the optimization of sequence alignment. In fact, many heuristic algorithms [18], [19], [20], including progressive and iterative refinement methods, are based on the DP. The alignment of two sequences can be implemented with DP [17], [21], [22] in $O(N^2)$ time and space complexity, where N is the length of the aligning sequences. As far as MSA is concerned, the run-time and space complexity grow exponentially with the number of sequences, indicating that the task for aligning many sequences of protein and amino acid will be very difficult [2].

In order to reduce the run-time and space complexity of DP, an efficient algorithm has been proposed in an MSA program, called multiple alignment using FFT (MAFFT). The program drastically reduces the execution time compared with existing methods [14], [23] by using fast Fourier transform (FFT) and cyclic convolution to identify homologous regions of sequences, whereas the accuracy of the resultant alignments is comparable with that of the most accurate methods in the literature. Although it is well-known that there is a strong relation between FFT and convolution, MAFFT is the first and only one to handle MSA by using

convolution and FFT. MAFFT is the fastest program for MSA compared with the existing programs whose accuracy of resulting alignments are comparable with the most accurate methods. The key technique in MAFFT, i.e., the identification of the homologous regions of aligning sequences by using FFT and convolution, can be applied to other algorithms of MSA. It is thus of interest to modify the algorithm with less memory size, lower computational complexity, more homologous regions detected, and higher efficiency.

The aim of this work is to improve MAFFT in efficiency. We modify the MAFFT, and devise a correlation computation algorithm and an FFT algorithm. In the former, an amino acid residue and a nucleotide residue are expressed as one and two complex numbers, respectively, instead of vectors consisting of two and four real numbers, in the MAFFT. The idea of linear convolution is utilized for improving the correlation computation between sequences by the proposed algorithm. The complex number scheme for nucleotide sequences has already been explained in the URL: http://mafft.cbrc.jp/alignment/software/algorithms/algorithms.html [24] and used in [25]. The modification in MAFFT is made after the publication of [26], and the Katoh2002 scheme (four real numbers are used for denote a nucleotide) is still used when the –nomemsave option is given. We rediscover and present the scheme in this paper. Our presentation contains not only the scheme for nucleotide sequences but also an exact derivation and the scheme for amino acid sequences. The correlations of alignment sequences are efficiently calculated using the proposed FFT algorithm which can eliminate the permutation of order. The elimination of order permutation can reduce the FFT CPU time since the time spent in the order permutation is about 15-25 percent of total FFT time. Moreover, it corresponds to a novel FFT algorithm on real data, which requires only the real parts of the final outputs of the correlations of sequences. The proposed correlation computation and FFT schemes allow us to achieve the improvement of MAFFT in efficiency.

The rest of this paper is organized as follows. Section 2 reviews MSA presented in MAFFT. Section 3 proposes a correlation calculation algorithm based on complex-valued expressions for bioinformatic sequences. Section 4 presents an FFT algorithm for the implementation of the proposed correlation calculation algorithm. Section 5 analyzes the performance of the two proposed algorithms by comparing them with those in MAFFT. The analysis and comparison include the required memory size, computational complexity, and the access to the lookup table of twiddle factors. Section 6 includes two simulations which simulate the computation of MSA and the computation of the correlation between two sequences. Finally, conclusions are drawn in Section 7.

2 Optimization of Aligning Path

In this section, we present two aspects of the optimization of the aligning path: digital sequence expression and correlation computation. These two aspects are basics for us to understand the proposed scheme. The presentation is derived from [26] and source code of the current MAFFT 7.127b.

The frequency of amino acid expressions strongly depends on the difference of physico-chemical properties, particularly volume and polarity, between the amino acid pair involved in the expression [27]. Expressions between physico-chemically similar amino acids tend to preserve the structure of proteins, and such neutral expressions have been accumulated in molecules during evolution [28]. It is reasonable to consider that an amino acid **a** is a vector consisting of the volume $v(\mathbf{a})$ and polarity $p(\mathbf{a})$, which is introduced by Grantham [29]. The values of the properties in amino acid are listed in Table 1.

TABLE 1
Values for Properties in Amino Acid [29]

Amino acid	Property		Amino acid	Property	
	p	v		p	v
Ser S	9.2	32.0	Tyr Y	6.2	136.0
Arg R	10.5	124.0	Cys C	5.5	55.0
Leu L	4.9	111.0	His H	10.4	96.0
Pro P	8.0	32.5	Gln Q	10.5	85.0
Thr T	8.6	61.0	Asn N	11.6	56.0
Ala A	8.1	31.0	Lys K	11.3	119.0
Val V	5.9	84.0	Asp D	13.0	54.0
Gly G	9.0	3.0	Glu E	12.3	83.0
Ile I	5.2	111.0	Met M	5.7	105.0
Phe F	5.2	132.0	Trp W	5.4	170.0

We now take the amino acid sequence s: $YKFLSCVSNM$ as an example. By looking up the table, the sequence s can be expressed as ((6.2, 136.0), (11.3, 119.0), (5.2, 132.0), (4.9, 111.0), (9.2, 32.0), (5.5, 55.0), (5.9, 84.0), (9.2, 32.0), (11.6, 56.0), (5.7, 105.0)). Then the sequence is converted to the normalized forms: $\hat{v}(a) = [v(\mathbf{a}) - \overline{v}]/\delta_v$ and $\hat{p}(\mathbf{a}) = [p(\mathbf{a}) - \overline{p}]/\delta_p$, where the overbar denotes the average of 20 amino acids ($\overline{v} = 84.025$ and $\overline{p} = 8.325$), and δ_v and δ_p denote standard deviations of volume and polarity respectively ($\delta_v = 41.86540189$ and $\delta_p = 2.622379645$). An amino acid sequence of symbols is then converted to a sequence of vectors.

The correlation between two sequences is defined as follows:

$$c(k) = c_v(k) + c_p(k), \quad -L + 1 \le k \le L - 1, \tag{1}$$

where $c_v(k)$ and $c_p(k)$ are the correlations of volume component and polarity component between these two sequences, M and N are the lengths of sequences 1 and 2, $L = \max(M, N)$, and k is the lag index. (In MAFFT, $L = 2^{\lceil \log_2 L \rceil}$, where $\lceil x \rceil$ rounds up the real number x to the nearest integer). The correlation $c(k)$ represents the degree of similarity of two sequences with the positional lag of k sites. The 20 highest values of $c(k)$ indicate that the sequences with the corresponding lag k may have homologous regions.

The correlation $c_v(k)$ of component volume between sequences 1 and 2 with the positional lag k is defined as:

$$c_v(k) = \sum_{m=1}^{M} \hat{v}_1(m)\hat{v}_2(m+k), \quad -L + 1 \le k \le L - 1, \tag{2}$$

where $\hat{v}_1(n)$ and $\hat{v}_2(n)$ are volume components of nth site of sequence 1 with length M and sequence 2 with length N respectively. Generally speaking, $M \simeq N$ in many cases, the calculation of Eq. (2) requires $O(MN)$ operations. Similar to Eq. (2), the correlation $c_p(k)$ of component polarity between sequence 1 and 2 with lag k is defined as

$$c_p(k) = \sum_{m=1}^{M} \hat{p}_1(m)\hat{p}_2(m+k), \quad -L+1 \le k \le L-1. \tag{3}$$

From the mathematical point of view, the lag k is in the range from $-L+1$ to $L-1$. However, the range of lag k in Eq. (3) is inaccurate from the biological viewpoints, since sequences are often aligned sequentially by matching the front to the front and the back to the back. It is impossible for a pairwise alignment to match the first residue of one sequence to the last residue of another one.

MAFFT computes Eq. (2) through FFT, which reduces the computational complexity from $O(L^2)$ to $O(L\log_2^L)$. The method is based on the cyclic convolution property of discrete Fourier transform (DFT):

$$V_1 \odot V_2 = IDFT(DFT(V_1)DFT(V_2)), \tag{4}$$

where V_1 and V_2 are the volume component vectors of a pairwise sequences, $\odot$ denotes cyclic convolution, and $DFT(V)$ and $IDFT(V)$ represent the DFT and inverse discrete Fourier transform (IDFT) of sequence V respectively. If the order of sequence 2 is reversed, Eq. (2) can be re-expressed as follows:

$$c_v(k) = \sum_{m=1}^{L} \hat{v_1}(m)\hat{\overleftarrow{v_2}}(L-k-m), \quad -L+1 \le k \le L-1, \tag{5}$$

where $\hat{\overleftarrow{v_2}}$ denotes the reversed sequence 2. Since Eq. (5) is the convolution representation of the correlation $c(k)$ and satisfies the condition of Eq. (4), the right part in Eq. (5) can be computed by using Eq. (4). If $DFT(V)$ or $IDFT(V)$ is evaluated with FFT, the computational complexity for computing Eq. (2) or (5) is proportional to $L\log_2 L$. The correlation $c_p(k)$ in Eq. (3) can be computed with the method similar to the correlation $c_v(k)$ in Eq. (2).

According to the 20 highest values of the correlation $c(k)$, MAFFT can detect and locate homologous regions, then a segment-level DP is conducted and obtains an optimal path of the alignment. By using the optimal path, the aligning matrix is divided into several sub-matrices at the boundary corresponding to the center of homologous segments, which can reduce the solution space of the alignment and save the CPU time [26].

The procedure of the one-to-one alignment can be extended to group-to-group alignment by considering the one-to-one alignment as the special case of the group-to-group alignment with one sequence in each group. The group-to-group alignment algorithm is a straightforward extension of the one-to-one alignment algorithm, and is the core of progressive and iterative approaches. The essential difference of group-to-group alignment from pairwise sequence alignment is the existence of gaps within each group of pre-aligned sequences [30]. By replacing $\hat{v}_1(m)$ and $\hat{v}_2(m)$ in Eq. (2) with $\hat{v}_{group1}(m)$ and $\hat{v}_{group2}(m)$, and $\hat{p}_1(m)$ and $\hat{p}_2(m)$ in Eq. (3) with $\hat{p}_{group1}(m)$ and $\hat{p}_{group2}(m)$, the two equations are extended to group-to-group alignment. The volume $\hat{v}_{group}(m)$ and polarity $\hat{p}_{group}(m)$ of a group are defined as:

$$\hat{v}_{group}(m) = \sum_{i \in group} W_i v_i(m), \quad m = 1, 2, \ldots, L, \tag{6}$$

and

$$\hat{p}_{group}(m) = \sum_{i \in group} W_i p_i(m), \quad m = 1, 2, \ldots, L, \tag{7}$$

where W_i is the weight factor of sequence i, which is evaluated in the same manner as in [10], [23], [31] for progressive method, or in the same manner as the weight system in [16], and L is the longest length in the group.

The correlation $c(k)$ between two nucleotide sequences can be evaluated through

$$c(k) = c_A(k) + c_T(k) + c_G(k) + c_C(k), \quad -L+1 \le k \le L-1, \tag{8}$$

by representing a nucleotide sequence as a sequence of 4-D vectors whose components are frequencies of A, T, G, and C at each column, instead of volume and polarity values. For example, the nucleotide sequence "*TAGTAA*..." can be expressed as ((0,1,0,0), (1,0,0,0), (0,0,1,0), (0,1,0,0), (1,0,0,0), (1,0,0,0), ...).

3 Proposed Correlation Computation

In this section, we propose a method for computing the correlation between sequences in MAFFT. The proposed method is different from the original method scheme in two aspects. (i) The method uses one complex data to express an amino acid residue, instead of a vector composed of two real numbers, and uses two complex numbers replacing a vector of four real numbers for expressing a nucleotide residue, (ii) Linear convolution with a limitation is proposed for computing the sequence correlations. For general mathematical sequences, the linear convolution can identify more homologous regions than those of the cyclic convolution. However, the bioinformatics sequences are just a very small part of the mathematical sequences. Moreover, the alphabets in a bioinformatics sequence are arranged in a methodical manner rather than a random fashion. Thus, the limitation of the linear convolution is proposed for improving the efficiency of the correlation computation of bioinformatic sequences of MAFFT. Although the convolution in the proposed algorithm is an intermediate form between cyclic convolution and linear convolution, we call it linear convolution.

3.1 One-to-One Alignment

From Section 2, we find that the computations of Eqs. (2) and (3) are not exactly identical to that of their cyclic convolution numerically. The computation using linear convolution is exactly identical to that of Eqs. (2) and (3). This is the reason why linear convolution in the proposed scheme replaces cyclic convolution in computing the correlations between sequences. Fig. 1 illustrates the computation of the correlation of sequence $s_1 =$ "$ABCD$" and sequence $s_2 =$ "$CABD$". In Fig. 1a, the correlation of the two sequences is computed directly according to Eqs. (2) and (3). Since the lengths of the two sequences are the same, the value of L in Eqs. (2) and (3) is four. The computation of the correlations

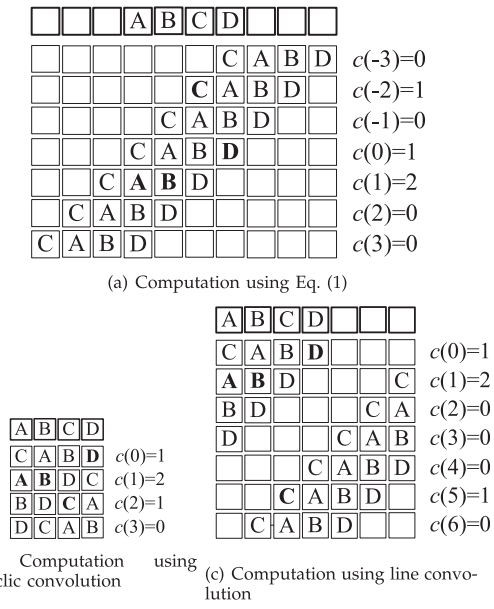


Fig. 1. Correlation computation of three methods.

using cyclic convolution is shown in Fig. 1b, where the correlation $c(k)$ is the number of the matched characters between these two sequences. It is clear that, the computation of the correlation using cyclic convolution does not keep pace with the computation directly using Eqs. (2) and (3), and some computations that need to be performed are neglected. Fig. 1c shows the computation of the correlations of these two sequences through linear convolution. By comparing Figs. 1a and 1b with Fig. 1c, one can find that the computation using linear convolution overcomes the deficiency of that of cyclic convolution in Figs. 1a and 1b. Moreover, more correlations obtained by linear convolution will result in more homologous segments identified.

An amino acid **a**, as mentioned before, can be represented as a vector whose components are the volume $v(\mathbf{a})$ and polarity $p(\mathbf{a})$. A complex data is like a vector of two real numbers except that it has special rules for addition and multiplication. In order to evaluate the convolution more efficiently, the proposed one-to-one alignment uses a complex data to describe an amino acid, i.e., one can use $\hat{p}(\mathbf{a}) \pm j\hat{v}(\mathbf{a})$ or $\hat{v}(\mathbf{a}) \pm j\hat{p}(\mathbf{a})$ to denote the amino acid **a**, where $j = \sqrt{-1}$.

The expressions of the aligning sequences should stay the same. We now consider the case of two sequences. Assume that the two sequences are s_1 and s_2. First, if the volume value (or polarity value) of an amino acid in s_1 is the real part of the expression, the volume value (or polarity value) of an amino acid should be also the real part of the expression in s_2. Second, if the imaginary part of an amino acid in s_1 is positive, the imaginary part of an amino acid in s_2 should be negative, and vice versa. The situation of multiple sequence alignment is similar to that of two sequences. The correlation $c(k)$ of the sequences s_1 and s_2 in Eq. (1) can be re-defined as

$$c(k) = \sum_{m=0}^{M+N-1} s_1(m) \sqcup s_2(m+k), \quad -L+1 \le k \le L-1, \tag{9}$$

where

$$\begin{aligned} & s_1(m) \sqcup s_2(m+k) \\ &= \Re\{s_1(m)\}\Re\{s_2(m+k)\} + \Im\{s_1(m)\}\Im\{s_2(m+k)\} \\ &= \hat{p}_1(m)\hat{p}_2(m+k) + \hat{v}_1(m)\hat{v}_2(m+k), \end{aligned} \tag{10}$$

and $\Re\{s(a)\}$ and $\Im\{s(a)\}$ denote the real and imaginary parts of the complex number $s(a)$ respectively. As the discussed in Section 2, it is impossible for a pairwise alignment to match the first residue of one sequence to the last residue of another one. Thus, it is unnecessary to obtain all $c(k)$ for Eq. (9) by padding enough zeros. To obtain $2\alpha+1$ reliable correlation $c(k)$, 2α zeros have to be padded for these two sequences, where $-\alpha \le k \le \alpha$ and $\lfloor (2L-N-M)/2 \rfloor \le \alpha \le (N+M-1)/2$, where $\lfloor x \rfloor$ denotes the biggest integer which is smaller than x. By padding $\lfloor (2\alpha+N-M+1)/2 \rfloor$ zeros at the end of the sequence s_2 and then reversing it, and padding $\lfloor 2\alpha-N+M+1)/2 \rfloor$ zeros at the end of the sequence s_1, a novel equation which is similar to but not a convolution is obtained as follows:

$$c(k) = \sum_{n=1}^{\beta} s_1(n) \sqcup s_2(k-n), \quad k = 0, 1, \ldots, \beta-\alpha, \beta-\alpha+1, \ldots, \beta-1, \tag{11}$$

where $\beta = \lfloor (2\alpha+N+M+1)/2 \rfloor$. In Eq. (11), the $\beta - 2\alpha - 1$ correlation $c(k)$ when $\alpha < k < \beta - \alpha$ are unnecessary. We call Eq. (11) semi-convolution, which is not a full convolution equation, since the operation operator is "$\sqcup$" rather than a multiplication. However, Eq. (11) can be computed efficiently by using standard convolution as

$$\begin{aligned} c(k) = \sum_{n=1}^{\beta} \Big(& s_1(n)s_2(k-n) \\ & - j[v_1(n)p_2(k-n) - v_2(k-n)p_1(n)] \Big) \\ = \; & IDFT\big(DFT(s_1)DFT(s_2)\big) \\ & - jIDFT\big(DFT(v_1)DFT(p_2) - DFT(v_2)DFT(p_1)\big), \\ & k = 0, 1, \ldots, \beta-\alpha, \beta-\alpha+1, \ldots, \beta-1, \end{aligned} \tag{12}$$

where $DFT(s)$ and $IDFT(s)$ represent the DFT and iDFT of the sequence s respectively. Since $v_1(n)$, $v_2(n)$, $p_1(n)$, and $p_2(n)$ are real numbers, the entries in $IDFT(DFT(v_1)DFT(p_2) - DFT(v_2)DFT(p_1))$ are real numbers and those of $jIDFT(DFT(v_1)DFT(p_2) - DFT(v_2)DFT(p_1))$ are imaginary numbers. Moreover, it is known that the values of $c(k)$ are real numbers. Therefore, in Eq. (12), the real parts of $IDFT(DFT(s_1)DFT(s_2))$ are equal to the values of $c(k)$, and the evaluation of the term $-jIDFT[DFT(v_1)DFT(p_2) - DFT(v_2)DFT(p_1)]$ can be neglected, i.e.,

$$c(k) = \Re\{IDFT(DFT(s_1)DFT(s_2))\}, \quad k = 0, 1, \ldots, \beta-\alpha, \beta-\alpha+1, \ldots, \beta-1, \tag{13}$$

In MAFFT, cyclic convolution is utilized for computing the correlation $c_v(k)$ in Eq. (2) and $c_p(k)$ in Eq. (3), and is implemented with a radix-2 FFT which taken from Flannery et al. [32]. Sequences have to been padded with zeros till their lengths reach powers-of-two, which makes its cyclic convolution like linear convolution in some extent.

In the next section, an FFT algorithm is proposed to efficiently compute the correlation $c(k)$ between the two sequences according to Eq. (13). In the algorithm, two FFT programs are developed. The purpose of one of the two programs is to compute DFTs of sequences and the second is to compute the IDFTs.

3.2 Extension to Group-to-Group Alignment

Similar to MAFFT, the proposed one-to-one alignment can also be easily extended to group-to-group alignment by considering Eq. (9) as the special case of one group with one sequence. If the terms $s_1(m)$ and $s_2(m+k)$ are replaced by $s_{group1}(m)$ and $s_{group2}(m+k)$, one can evaluate the correlation between two groups through Eq. (9). The expression of the group s is defined as

$$\hat{s}_{group}(n) = \sum_{i \in group} W_i s_i(n), \quad n = 1, 2, \ldots, L-1, \tag{14}$$

where L is the maximum length of sequences among the group.

If the method is applied to nucleotide sequences, the vector of four real numbers of a nucleotide is to be converted to two complex numbers in which each complex number derives from two of the four frequencies of A, T, G, and C at each column. Assume that the frequencies of A and T form a complex number and those of G and C form another one. The correlation $c(k)$ between two nucleotide sequences of s_1 with length M and s_2 with length N is defined as

$$\begin{aligned} c(k) &= c_{AT}(k) + c_{GC}(k) \\ &= \Re\{IDFT(DFT(s_{AT1})DFT(s_{AT2}))\} \\ &\quad + \Re\{IDFT(DFT(s_{GC1})DFT(s_{GC2}))\}, \\ &\quad k = 0, 1, \ldots, \beta-\alpha, \beta-\alpha+1, \ldots, \beta-1, \end{aligned} \tag{15}$$

which is similar to Eq. (13). The convolutions in Eqs. (13) and (15) will be evaluated with the proposed FFT algorithm in Section 4.

We now summarize the proposed algorithm for the correlations between sequences. The first and most important thing is to express the amino acid and the nucleotide in a regular and strict way. Second, the linear convolution of the sequences will be efficiently implemented with the proposed FFT algorithm in Section 4. The real parts of the linear convolution are the final outputs. Through the correlations in Eqs. (13) and (15), as mentioned in Section 2, we can obtain the homologous regions of pairs of sequences or sequence groups. The homologous regions divide the 2-D array constructed from the aligning sequences or sequence groups into some sub-arrays, while the alignment of two long sequences of sequence groups is decomposed into some sub-alignments of pairs of shorter sequences or sequence groups. The resultant sub-alignments can be implemented with any approach of aligning two sequences or sequence groups. When the alignments of pairs of sequences or sequence groups are used by progressive method or other MSA methods, the alignment of multiple sequences is obtained efficiently.

4 Proposed FFT Algorithm

It is obligatory to implement three FFTs for a protein sequence alignment and six FFTs for a DNA sequence alignment. Two of the three FFTs or four of the six FFTs are implemented for computing the DFTs of sequences (or groups). The other FFTs are implemented for computing the iDFT of the product of the DFTs of the pairwise of sequences (or groups). An FFT algorithm is proposed for efficiently computing the DFTs and iDFTs. In the algorithm, two FFT programs are developed. One of the programs can implement the computation of the DFTs of sequences (or groups). Another one can implement the IDFT of the product of the DFTs. The two programs are all based on the split-radix FFT (SRFFT) algorithm [33], [34], [35]. The algorithm for DFTs is decimation-in-frequency (DIF) structure. The algorithm for IDFTs is decimation-in-time (DIT) structure, which is a novel output pruning and can delete all unnecessary operations since only the real parts of the outputs are necessary. Both algorithms eliminate the order permutation since this is redundant for the proposed sequence alignment. The time spent in the order permutation is about 15-25 percent of total FFT time. Thus, the elimination of order permutation can reduce the FFT CPU time.

4.1 Order Permutation Elimination

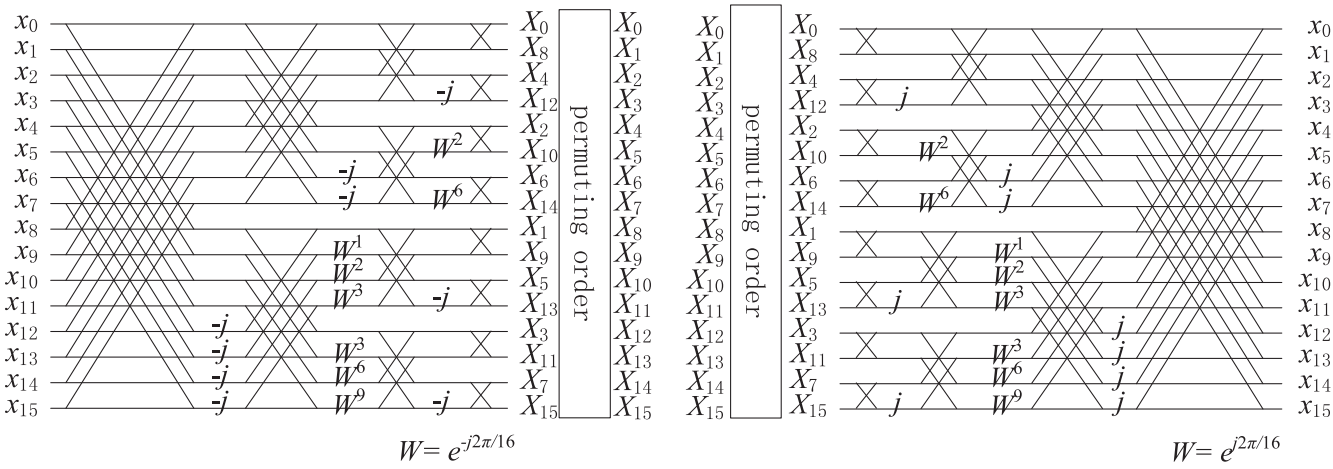
Permuting order in the implementation of FFTs does not require real operations. However, the striding access to memory makes a lot of demands on CPU time. Generally speaking, an FFT algorithm is used in applications where Fourier analysis is implemented in frequency domain, and it is necessary to permute order. Owing to non-existence of Fourier analysis of frequency domain in the proposed sequence alignment, permuting order is unnecessary for the proposed FFT implementation.

Therefore, in the proposed FFT implementation, the order permutation is omitted by blending an DIF SRFFT algorithm and an DIT SRFFT algorithm. As illustrated in Fig. 2, the implementation of the DIF SRFFT algorithm permutes the order of sequences at the end of programs, and the implementation of the DIT SRFFT algorithm permutes the order of sequences at the beginning of programs. In order to eliminate the order permutation, the proposed implementation method uses the DIF SRFFT algorithm to evaluate the DFTs of sequences, and then uses the DIT SRFFT algorithm to evaluate corresponding IDFTs.

The proposed FFT implementations are based on the SRFFT algorithm. The SRFFT algorithm is the best possible compromise between regularity and computational complexity [36], and has the optimal number of real multiplications and the best known minimum of real additions for lengths up to and including 16. It is the most popular FFT algorithm on general processors and is a good choice for the proposed method to implement its FFTs.

Let us review the definition of DFT and the decomposition of the SRFFT algorithm. For a length-L sequence, we can obtain a new sequence of length-$N = 2^{\lceil \log_2(L) \rceil}$ sequence $x(n)$ by padding zeros, its DFT is also a length N sequence,

$$X(k) = \sum_{n=0}^{N-1} x(n) W_N^{nk}, \qquad k = 0, 1, 2, \ldots, N-1, \tag{16}$$



(a) DIF SRFFT for 16-point DFTs (b) DIT SRFFT for 16-points iDFTs

Fig. 2. Flowgraph of 16-point DFT and IDFT.

where $W = e^{-j2\pi/N}$ and $j = \sqrt{-1}$. The DIF SRFFT algorithm is similar to the DIT SRFFT algorithm. The difference between DIF and DIT lies only in the direction of their flowgraph. The DIF decomposition of SRFFT gives

$$X(2k) = \sum_{n=0}^{N/2-1} (x(n) + x(n+N/2))W_N^{nk}, \quad k = 0, 1, 2, \ldots, N/2-1, \tag{17}$$

for the even-indexed terms, and

$$X(4k+1) = \sum_{n=0}^{N/4-1} ((x(n) - x(n+N/2)) - j(x(n+N/4) - x(n+3N/4)))W_N^n W_N^{nk}, \quad k = 0, 1, 2, \ldots, N/4-1, \tag{18}$$

and

$$X(4k+3) = \sum_{n=0}^{N/4-1} ((x(n) - x(n+N/2)) + j(x(n+N/4) - x(n+3N/4)))W_N^{3n} W_N^{nk}, \quad k = 0, 1, 2, \ldots, N/4-1, \tag{19}$$

for the odd-indexed terms. In the case of the DIT SRFFT algorithm, the decomposition is

$$X(k) = \sum_{n=0}^{N/2-1} x(2n)W_{N/2}^{nk} + W_N^k \sum_{n=0}^{N/4-1} x(4n+1)W_{N/4}^{nk} + W_N^{3k} \sum_{n=0}^{N/4-1} x(4n+3)W_{N/4}^{nk}, \quad k = 0, 1, 2, \ldots, N-1. \tag{20}$$

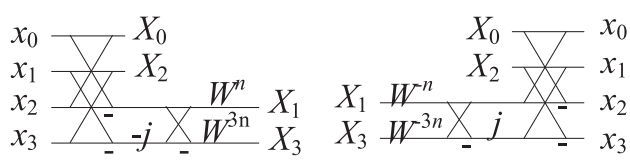
The flowgraph of DIF SRFFT for 16-point DFT is illustrated in Fig. 2a, and that of DIT SRFFT for 16-point IDFT is shown in Fig. 2b.

4.2 DIF and DIT Programs

The SRFFT algorithm decomposes a length-$N = 2^m$ DFT into a length-$N/2$ sub-DFT and two length-$N/4$ sub-DFTs by implementing two special butterflies of $n = 0$ and $n = N/8$, and $N/4 - 2$ general butterflies. To simplify butterfly analysis, we are going to focus on the general L-butterflies. There exist two types of general butterflies in the proposed FFT implementation, as shown in Fig. 3. The general butterfly illustrated in Fig. 3a is implemented in the DIF SRFFT algorithm that is developed for computing the DFTs of sequences. The general butterfly illustrated in Fig. 3b is implemented in the DIT SRFFT algorithm that is developed for computing the IDFTs of the product of the DFTs of two sequences.

The proposed FFT algorithm eliminates the order permutation. The algorithm includes two FFT programs. One of them is to compute the DFTs of sequences, which uses DIF structure of SRFFT. The other program is devised for computing the IDFT of the product of two DFTs, which uses the DIT structure of SRFFT. Among them, the latter only requires real outputs, and is a variant of the real split-radix IFFT. Compared with the standard SRFFT algorithm, the proposed IFFT algorithm does not need to permute their orders, and can save half of real operations. The simulation result shows that evaluations of the correlation between two sequences in the proposed manner saves CPU time ranging from 66.0 to 88.0 percent over that in MAFFT.

The program of the DIF SRFTT algorithm is similar to that of the DIT SRFFT algorithm, only the direction of control flow and the types of butterflies are different. Two three-loop structure programs are developed on Windows and Ubuntu in C/C++. The searching technique of the L-shape block adopts the method presented by Sorensen in et al. [34].



(a) General butterfly of DIF split-radix FFT (b) General butterfly of DIT split-radix inverse FFT

Fig. 3. General butterflies of proposed FFT algorithm.

To minimize the access to the lookup table of twiddle factors, two programs all breath-first traverse all the L-shape blocks. The programs stride $m-1$ stages in the outer loop. In the middle loop, the programs lookup the table for twiddle factor of one L-shape butterfly and prepare for the implementation of the butterfly in the inner loop. The programs implement one L-shape butterfly at each L-shape block while the programs traverses the block in the inner loop. The final stage for the DIF structure and the first stage for the DIT structure consist of length-2 DFTs. The length-4 L-shape butterfly is not proper for them.

5 Performance Analysis

In this section, we analyze the performance of the proposed algorithms by comparing with those in MAFFT. The analysis and comparison include the computational complexity, and the access to the lookup table of twiddle factors.

5.1 Computational Complexity

Assume the lengths of FFTs are N in the computation of the correlations of the aligning sequences. We now consider the computational complexities of the proposed scheme and MAFFT for the computation of the correlation of two sequences.

In MAFFT, an DFT or IDFT of length-N is implemented with a general radix-2 FFT program in the computational complexity of $5N\log_2 N - 10N + 16$. The FFT source code in MAFFT has been taken from [32]. The computations of correlations of two amino acid sequences contains the evaluations of four DFTs and two IDFTs of length-N. In addition, the computations contain $2N$ complex multiplications and N real additions. For the correlations of two nucleotide sequences, MAFFT requires the evaluations of eight DFTs and four IDFTs of length-N, $4N$ complex multiplications, and $3N$ real additions.

In the proposed algorithms, an DFT of length-N is implemented with the DIF SRFFT algorithm in the computational complexity of $4N\log_2 N - 6N + 8$, and an IDFT of length-N is implemented with the real data DIT SRFFT algorithm in the computational complexity $2N\log_2 N - 3N + 4$. For the correlations of two amino acid sequences, there are two DFTs and one IDFT of length-N needed to evaluate. In addition, the computations requires N complex multiplications. For the correlations of two nucleotide sequences, the computations contain the evaluations of four DFTs and two IDFTs of length-N, $2N$ complex multiplications, and N real additions.

Table 2 shows the computational complexities of the proposed scheme and MAFFT for the correlations of two sequences. We can see that the proposed scheme obtains a substantial reduction of computational complexity for the correlations of both two amino acid sequences and two nucleotide sequences.

5.2 Access to Lookup Table

In recent decades, the memory performance improvements have not kept pace with processor, and this trend is likely to continue. So, it becomes increasingly important for memory assess to be optimized on modern processors. There are few algorithms considering the access optimization to twiddle factors which arise in Cooley and Tukey's algorithm. The twiddle factors are a set of complex roots of unity, fixed by the transform order and the particular algorithm [37]. The breath-first Cooley and Tukey's traversing algorithms requires $O(N)$ access to twiddle factors, and [38] presents a more irregular algorithm requiring fewer than $N/2$, where $N = 2^m$ is the length of the DFT. These traversals require striding access to memory (sample data), which is more expensive than the sequential access to memory. However, by using some cache optimization strategies [39], this deficiency can be avoided. Bowers et al. [37] have proposed G^T- and G-based implementations requiring N access to twiddle factor and no such striding access to memory. However, these algorithms are only suitable for the radix-2 FFT with power-of-two DFTs. The two implementations in this work require $N-2$ real operations while the twiddles factors W^0 and $W^{N/8}$ being or not being initiated and stored in the registers of the processor. Their regularity is the same as that of the breath-first Cooley and Tukey's algorithm and more regular than that of [38].

TABLE 2
Computational Complexity of Correlation Computation of Two Sequences

	Computational complexity	Rounds of order permutation
2002 MAFFT		
Amino acid	$30N\log_2 N - 47N + 96$	6
Nucleotide	$60N\log_2 N - 93N + 192$	12
Newer MAFFTs		
Amino acid	$15N\log_2 N - 24N + 48$	3
Nucleotide	$60\ N\log_2 N - 93N + 192$	12
Proposed scheme		
Amino acid	$10N\log N - 10N + 20$	0
Nucleotide	$20N\log_2 N - 19N + 40$	0

6 Results

6.1 Simulations

In order to evaluate the performance of the proposed method, we conduct simulations which are focused on the algorithm efficiency and number of detected homologous regions, on an Ubuntu operating system (Intel Xeon E6700 3.2 GHz with 2 GB of memory). The gcc version 4.5.2 compiler is used with the optimization option '-O3'. We set five switch constants in mltaln.h in MAFFT source code to control five modifications that can affect MAFFT performance. The five switch constants are listed as follows:

- MOD.1 controls "fftWinSize" = 30 or = 100 for DNA sequence alignment. The value of the variable "fftWinSize" is the size of the sliding window.
- MOD.2 controls whether or not neglects the sentence in Falign.c, in the function "Falign()": "if(tmpint == 0) break;". In the loop of the identification of homologous regions from the 20 highest values of the correlations, this sentence control to exit the loop if the current correlation does not contains a homologous region.
- MOD.3 controls whether or not add MAXLAG/2 to the alignment length "nlen" in Falign() in Falign.c.

TABLE 3
Number of Detected Homologous Regions by MAFFT with Cyclic or Linear Convolution

Length	DNA01 (fftWinSize = 30)		DNA02 (fftWinSize = 100)		DNA03 (fftWinSize = 100)		Protein (fftWinSize = 20)	
	Cyclic	Linear	Cyclic	Linear	Cyclic	Linear	Cyclic	Linear
128	2/2	1/2	0/0	0/0	0/0	0/0	1/1	2/2
256	2/2	2/3	0/0	0/0	1/1	1/1	2/2	4/4
512	0/2	2/3	0/1	0/1	3/3	3/3	1/2	4/4
1,024	1/7	7/8	0/1	0/3	0/0	0/0	6/8	11/17
2,048	1/1	1/5	9/9	12/12	9/9	9/9	5/13	7/19
4,096	0/3	2/5	0/14	24/24	1/1	1/2	0/19	29/33
8,192	5/12	5/15	25/26	25/32	48/48	48/49	7/13	12/21
16,384	53/62	62/64	37/50	37/52	80/80	80/84	36/44	55/59
32,768	0/34	37/38	30/73	65/83	36/38	36/38	44/100	117/117
Total	64/128	121/141	101/174	163/207	178/180	178/186	102/202	241/276

MAXLAG/2 is derived from the variable "α" of the proposed linear convolution scheme. In the modified MAFFT, MAXLAG/2 is 75.

- MOD.4 controls the possible candidate lags whether or not in the range from -MAXLAG/2 to MAXLAG/2.
- MOD.5 controls whether or not use the new FFT program.

In order to obtain an optimal performance, several modifications may be combined in a revision. The MAFFT with the optimal combination of modification 1, 4 and 5 is the so-called modified MAFFT in the paper. For a protein sequence alignment, the modification 1 is neglected automatically by the modified MAFFT.

By ROSE program [40], [41], we generate the two datasets "DNA01" and "Protein" with $Relatedness = 10$, and the two datasets "DNA02" and "DNA03" with $Relatedness = 20$. Every dataset contains nine pairs of sequences whose lengths are from 128 to 32,768. From the results based on datasets "DNA01" and "DNA02", it is demonstrated that our proposed linear convolution scheme can detect more homologous regions for DNA sequence alignment. The dataset "DNA03" is a general DNA sequence dataset to demonstrate the general performance of our proposed scheme. The dataset "Protein" is a protein sequence dataset, which can demonstrate the advantage that our proposed linear convolution scheme can detect more homologous regions for protein sequence alignment.

Table 3 shows the number of the identified homologous regions for the four datasets "DNA01", "DNA02", "DNA03" and "Protein" by MAFFT with cyclic or linear convolution. For the dataset "DNA01", the sliding window sizes of MAFFT with cyclic or linear convolution are all 30 sites. For the datasets "DNA02" and "DNA03", the sliding window sizes of MAFFT with cyclic or linear convolution are all 100 sites, i.e., the default sliding window size of the original MAFF for DNA sequences. For the dataset "Protein", the sliding window sizes of MAFFT with cyclic or linear convolution are all 20 sites, i.e., the default sliding window size of the original MAFFT for protein sequence alignment. The sliding window size for DNA sequence alignment can be 30 or 100 sites by setting the switch constant "MOD.1" to "0" or "1". MAFFT with linear convolution should set the switch constant "MOD.3" to "1", i.e., to extend the lengths of aligning sequences to reach the length of linear convolution. The switch constant "MOD.3" in MAFFT with cyclic convolution should be "0". The values in Table 3 are the final values of the variable "count" in the function "Falign()" in the C ++ program "Falign.c" for different lengths of MAFFT with cyclic or linear convolution, i.e., the total number of detected homologous regions. For the two values of MAFFT with cyclic or linear convolution of each sequence length of each dataset, the first one can be obtained when the switch constant "MOD.2" is "0", and the second one can be obtained when the switch constant "MOD.2" is "1".

From the mathematical point of view, the number of the identified homologous regions depends on the way whether the correlations are computed using linear convolution or cyclic convolution. The cyclic convolution will result in an incomplete computation of correlations between aligning sequences, as discussed in Section 3.1. When the sequences in the datasets "DNA01", "DNA02" and "Protein" are generated by ROSE, only those are detected more homologous regions with the proposed linear convolution are kept. The experimental results show that MAFFT with linear convolution can identify homologous regions twice those of cyclic convolution for these three datasets.

From the bioinformatics point of view, the alphabets in the sequences are arranged in a methodical manner rather than a random fashion. Thus, for a general sequence alignment, the effect of the linear convolution will be not so obvious like the datasets "DNA01", "DNA02" and "Protein". The dataset "DNA03" contains a set of general DNA sequences generated by ROSE program [40], [41]. When the switch constant "MOD.2" is "0", the number of detected homologous regions by MAFFT with linear convolution is the same as that of MAFFT with cyclic convolution. When the switch constant "MOD.2" is "1", and the number of detected homologous regions by MAFFT with linear convolution is six more than that of MAFFT with cyclic convolution. This result can show the general performance of our proposed scheme in detecting homologous regions.

Besides the number of detected homologous regions, the proposed scheme can affect the efficiency of the region-level DP by the parameter α in Eq. (11). The simulations estimate the efficiency in CPU time of the proposed scheme. Tables 4, 5, 6 and 7 give respectively the CPU time of the four datasets. The time in Tables 4, 5, 6 and 7 is the CPU

TABLE 4
CPU Time for DNA Sequences of the Dataset "DNA01"

Length	Original MAFFT (A)	MAFFT with one separate modification					MAFFT with modifications		(A−B)/B (%)
		MOD.1	MOD.2	MOD.3	MOD.4	MOD.5	1 and 4 (C)	1, 4 and 5 (B)	
128	0.001389	0.001546	0.002285	0.001572	0.001417	0.001351	0.001169	0.001361	2.06%
256	0.001134	0.004628	0.006249	0.004589	0.004267	0.004247	0.004332	0.004443	−74.48%
512	0.015931	0.016134	0.019815	0.01684	0.016097	0.016244	0.006176	0.006069	162.50%
1,024	0.055985	0.053899	0.067599	0.061342	0.060380	0.016710	0.013661	0.013470	315.63%
2,048	0.117004	0.092274	0.100446	0.110018	0.071478	0.064984	0.081252	0.044558	162.59%
4,096	0.309503	0.279974	0.348462	0.282558	0.303333	0.312860	0.129712	0.164499	88.15%
8,192	1.203741	0.308130	0.598595	1.177470	1.123019	1.136245	0.350047	0.267397	350.17%
16,384	1.227750	0.551834	1.201293	1.180107	1.198868	1.207881	0.525641	0.457826	168.17%
Total	2.932437	1.308419	2.344744	2.834496	2.778859	2.760522	1.111990	0.959623	205.58%

TABLE 5
CPU Time for DNA Sequences of the Dataset "DNA02"

Length	Original MAFFT (A)	MAFFT with one separate modification					MAFFT with modifications		(A−B)/B (%)
		MOD.1	MOD.2	MOD.3	MOD.4	MOD.5	1 and 4 (C)	1, 4 and 5 (B)	
128	0.001454	0.001223	0.002380	0.001555	0.001502	0.001477	0.001256	0.001098	32.42%
256	0.004458	0.001223	0.006369	0.004671	0.004476	0.004390	0.004480	0.002250	98.13%
512	0.015343	0.010697	0.013929	0.015806	0.015275	0.015394	0.005793	0.004222	263.41%
1,024	0.058716	0.023127	0.044397	0.059570	0.023259	0.058551	0.012112	0.018256	221.63%
2,048	0.028103	0.018053	0.041607	0.058127	0.028346	0.016519	0.017888	0.017821	57.70%
4,096	0.262949	0.269357	0.118905	0.209513	0.079892	0.255548	0.049850	0.046833	461.46%
8,192	0.318229	0.230719	0.345677	0.326979	0.305558	0.308853	0.207477	0.147998	115.02%
16,384	1.207311	0.686704	0.643782	1.176340	1.159312	1.161829	0.225703	0.168733	615.52%
Total	1.896563	1.241103	1.217046	1.852561	1.617620	1.822561	0.524559	0.407211	365.74%

TABLE 6
CPU Time for DNA Sequences of the Dataset "DNA03"

Length	Original MAFFT (A)	MAFFT with one separate modification					MAFFT with modifications		(A−B)/B (%)
		MOD.1	MOD.2	MOD.3	MOD.4	MOD.5	1 and 4 (C)	1, 4 and 5 (B)	
128	0.001400	0.001056	0.002317	0.002259	0.001405	0.001408	0.001044	0.000302	363.58%
256	0.003290	0.002250	0.004142	0.004836	0.002336	0.002345	0.002219	0.002170	51.61%
512	0.004070	0.004331	0.007429	0.007256	0.003969	0.003998	0.004243	0.004258	−4.42%
1,024	0.060661	0.01224	0.018181	0.028259	0.039354	0.060623	0.018390	0.018101	235.13%
2,048	0.030596	0.019551	0.042238	0.042297	0.030370	0.055439	0.019273	0.019250	58.94%
4,096	0.207959	0.064030	0.235004	0.249427	0.216534	0.217959	0.099573	0.074558	178.92%
8,192	0.094544	0.039005	0.052383	0.069707	0.075640	0.024645	0.087496	0.045547	107.57%
16,384	0.166922	0.148517	0.137867	0.113686	0.081787	0.103309	0.117290	0.080412	107.58%
Total	0.569442	0.290980	0.499561	0.517727	0.451395	0.469726	0.349528	0.244598	132.81%

TABLE 7
CPU Time for Protein Sequences of the Dataset "Protein"

Length	Original MAFFT (A)	MAFFT with one separate modification				Modified MAFFT (B)	(A−B)/B (%)
		MOD.2	MOD.3	MOD.4	MOD.5		
128	0.000872	0.001841	0.001404	0.000902	0.000922	0.001218	−28.41%
256	0.001852	0.003652	0.002116	0.001807	0.001845	0.001617	14.53%
512	0.005425	0.008088	0.005767	0.004150	0.005453	0.004610	17.68%
1,024	0.005570	0.034733	0.017446	0.010314	0.010615	0.010374	−46.31%
2,048	0.029323	0.076400	0.024328	0.076035	0.029054	0.057397	−48.91%
4,096	0.249468	0.063404	0.128520	0.080873	0.221190	0.073178	240.91%
8,192	0.305795	0.445294	0.105022	0.099321	0.274955	0.173370	76.38%
16,384	0.665334	0.573191	2.481259	0.221617	0.599801	0.286970	131.85%
Total	1.263639	1.206603	2.765862	0.495019	1.143835	0.608734	107.58%

TABLE 8
Accuracy of Different Categories of BAliBASE3.0

Method	RV11 Q/TC	RV12 Q/TC	RV20 Q/TC	RV30 Q/TC	RV40 Q/TC	RV50 Q/TC	average Q/TC
MAFFT-FFT-NS-1	0.598/0.340	0.927/0.779	0.967/0.361	0.811/0.332	0.820/0.451	0.848/0.511	0.812/0.439
MAFFT-FFT-NS-2	0.603/0.382	0.974/0.786	0.979/0.370	0.819/0.475	0.884/0.530	0.853/0.525	0.829/0.525
MAFFT-FFT-NS-i	0.615/0.395	0.982/0.796	0.989/0.382	0.832/0.490	0.899/0.547	0.864/0.539	0.841/0.538
NEW-FFT-NS-1	0.598/0.340	0.927/0.779	0.967/0.361	0.811/0.332	0.820/0.451	0.848/0.511	0.812/0.439
NEW-FFT-NS-2	0.603/0.382	0.974/0.786	0.979/0.370	0.819/0.475	0.884/0.530	0.853/0.525	0.829/0.525
NEW-FFT-NS-i	0.615/0.395	0.982/0.796	0.989/0.382	0.832/0.490	0.899/0.547	0.864/0.539	0.841/0.538

time required in one execution of the function "Falign()" in the program "Falign.c" of MAFFT. Every result is the CPU time of one execution of one alignment. Although the results contain some uncertainties, the trend of the results is clear. By comparing the CPU time of the original MAFFT to that of MAFFT with five separate modifications or the modified MAFFT, we can see that the separate modifications 1, 4, and 5 can improve slightly the efficiency of MAFFT in CPU time, and the separate modifications 2 and 3 will impair the efficiency of sequence alignment of MAFFT in CPU time, but the combination of the modifications 1, 4 and 5 can greatly improve the efficiency of MAFFT in CPU time for DNA sequence alignment. The combination of modifications 1 and 4 requires CPU time more than the combination of the modifications 1, 4 and 5. The combination of modifications 4 and 5 can greatly improve the efficiency of MAFFT in CPU time for protein sequence alignment.

Considering Tables 3, 4, 5, 6 and 7 together, we can draw several interesting conclusions. (i) By extending the lengths of sequences, the linear convolution can identify more homologous regions. However, this separate modification impairs the performance of MAFFT in CPU time, since the computation of the extended FFT needs extra CPU time, and the identified homologous regions which are not detected by the original MAFFT are perhaps redundancy for the sequence alignment in bioinformatics; (ii) Similarly, although by setting switch constant "MOD.3" to 1, i.e., neglecting the sentence "if (tmpint == 0) break;" in the function "Falign()" in the program "Falign.c", MAFFT can identify more homologous regions, this modification also impairs the performance of MAFFT in CPU time in most cases; (iii) The parameter α of the proposed scheme, i.e., the limitation of the proposed linear convolution, is very useful for improving the performance of MAFFT in CPU time. A possible reason is that this parameter can improve the efficiency of region-level DP. (iv) To estimate the effect of the modification of the sliding window size on alignment accuracy, (the sliding window in the modified MAFFT is set to 30 sites rather than 100 sites for DNA sequence alignment), we compare the alignments of the original MAFFT with those of the modified MAFFT for our four designed datasets and BAliBASE 3.0 benchmark tests (RV11, RV12, RV20, RV30, RV40, and RV50), and find that the alignments of the modified MAFFT are fully identical to that of the original MAFFT, except for the one alignment of "BB20041" of BAliBASE 3.0 benchmark tests for MAFFT-FFT-NS1, and the three alignments of "BB20041", "BB30008" and "BB40035" of BAliBASE 3.0 benchmark tests for MAFFT-FFT-NS2. However, even for these four exceptions, their SP score and TC score are all the same. Thus, although we are not sure, but we want to say that it perhaps does not affect the alignment accuracy for the modified MAFFT to set the sliding window to 30 sites for DNA sequence alignment.

6.2 Experiment on BALiBASE

To benchmark our proposed algorithm for MSA, we carry out experiment by using BALiBASE 3.0, a standard database for MSA [42], [43]

We used the benchmark BAliBASE3.0 to assess the alignment accuracy of the modified algorithm in comparison with the original MAFFT program. Two accuracy measures are employed to score the alignment, i.e., the quality score (Q), which is the number of correctly aligned residue pairs divided by the number of residue pairs in the reference alignment, and total column score (TC), which is the number of correctly aligned columns divided by the number of columns in the reference alignment [3].

Tables 8 shows the accuracy of different categories of BAliBASE3.0. We can see that the modified program maintains identical accuracy to the original MAFFT, which verifies the validity of the proposed algorithm.

The original MAFFT FFT-NS-2 can save about 6-8 seconds for each BAliBASE 3.0 benchmark test (RV11, RV12, RV20, RV30, RV40, and RV50) on our platform. In original MAFFT, the aligning sequence is padded automatically with zeros, since their length has to reach to, power-of-two, the length of the FFT algorithm, which satisfies partially and even fully the configuration of the linear convolution. In addition, the data size of BAliBASE entries are small. Thus, the speed improved by the modified program is small on BAliBASE 3.0 benchmark tests. The real time of BAliBASE 3.0 tests is showed in Table 9. The time contains not only CPU time but also the time of other operations. The benchmark tests consist of RV11, RV12, RV20, RV30, RV40, and RV50.

7 Conclusion

We have developed a scheme for correlations computation between aligning sequences. The scheme uses one and two complex numbers to represent an amino acid residue and a nucleotide residue, respectively. The correlations and

TABLE 9
Real Time of BAliBASE 3.0 Benchmark Tests
(RV11, RV12, RV20, RV30, RV40, and RV50)

	FFT-NS-1 (s)	FFT-NS-2 (s)
Original MAFFT	45.719	92.954
Modified MAFFT	44.615	89.342

homologous regions between sequences can be obtained by using linear convolution with a limitation and sliding window analysis. The proposed correlation calculation alignment reduces half of the requirement of memory size and more than half of the real operations for computing the correlation between two sequences over the method in MAFFT. The proposed FFT algorithm eliminates the order permutation, and has the least access to the lookup table of twiddle factors. Moreover, the FFT algorithm is novel because it requires only real parts of output signals. Simulation results showed that, (i) in theory, the use of the linear convolution can detect the more homologous regions, which is particularly clear to the alignment length of powers-of-two. In practice, to reach to the length of FFT algorithm, power-of-two, the original MAFFT automatically pads the sequences with zeros, which satisfy partially or even fully the configuration of the linear convolution. In addition, from the bioinformatics point of view, the identified extra homologous regions are partly redundant for the alignment of the region-level DP, which may impair the performance of MAFFT in CPU time. Thus, the ability that the proposed linear convolution can identify more homologous regions and its effect are not as great as expected; (ii) modified MAFFT is 107.58 to 365.74 percent faster than the original MAFFT for one execution of the function Falign(), and its alignment accuracy remains unchanged for FFT-NS-1 and FFT-NS-2. The possible reason is that the limitation of the proposed linear convolution can improve greatly the performance of the region-level DP alignment in CPU time.

Appendix A
Supplementary Data

The source code is provided as supplementary material in the revised manuscript. The code is modified from the source code of MAFFT version 7.127b on Ubuntu in five aspects: (1) The size of the sliding window for DNA sequence alignment in the function "alignableReagion()" in the file "fftFunctions.c". (2) One of the configurations of linear convolution, i.e., the modification of the length of alignment in the file "Falign.c". (3) Count all possible homologous regions after the analysis of the sliding window in the "Falign()" in the file "Falign.c". (4) Another configuration of linear convolution, i.e., the configuration of α in Eq. (11) in the function "getKouho()" in the file "fftFunctions.c". (5) New FFT source code in the file "fft.c", that is compatible with the original MAFFT. These five modifications are controlled by five switch constants "MOD.1", "MOD.2", "MOD.3", "MOD.4", and "MOD.5" in the head file "mltaln.h". The configuration of linear convolution is an inexact and empirical evaluation, need to be improved in the future.

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References

[1] G. Vogt, T. Etzold, and P. Argos, "An assessment of amino acid exchange matrices in aligning protein sequences: The twilight zone revisited," *J. Molecular Biol.*, vol. 249, no. 4, pp. 816–831, 1995.
[2] L. Wang and T. Jiang, "On the complexity of multiple sequence alignment," *J. Comput. Biol.*, vol. 1, no. 4, pp. 337–348, 1994.
[3] S. Hosni, A. Mokaddem, and M. Elloumi, "A new progressive multiple sequence alignment algorithm," in *Proc. IEEE 23rd Int. Workshop Database Expert Syst. Appl.*, Vienna, Austria, Aug. 2012, pp. 195–198.
[4] J. Stoye, "Multiple sequence alignment with the divide-and-conquer method," *Gene*, vol. 211, no. 2, pp. GC45–GC56, 1998.
[5] M. Elloumi and A. Mokaddem, "A heuristic algorithm for the N-LCS problem," *J. Appl. Math., Stat. Informat.*, vol. 4, no. 1, pp. 17–27, 2008.
[6] S. R. Eddy, "Multiple alignment using hidden Markov models," in *Proc. 3rd Annu. Int. Conf. Intell. Syst. Molecular Biol.*, Jul. 1995, pp. 114–120.
[7] C. Notredame and D. G. Higgins, "SAGA: Sequence alignment by genetic algorithm," *Nucleic Acids Res.*, vol. 24, no. 8, pp. 1515–1524, 1996.
[8] R. C. Edgar and K. Sjölander, "SATCHMO: Sequence alignment and tree construction using hidden Markov models," *Bioinf.*, vol. 19, no. 11, pp. 1404–1411, 2003.
[9] R. K. Bradley, A. Roberts, M. Smoot, S. Juvekar, J. Do, C. Dewey, I. Holmes, and L. Pachter, "Fast statistical alignment," *PLoS Comput. Biol.*, vol. 5, no. 5, p. e1000392, 2009.
[10] J. D. Thompson, D. G. Higgins, and T. J. Gibson, "CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, Position-specific gap penalties and weight matrix choice," *Nucleic Acids Res.*, vol. 22, no. 22, pp. 4673–4680, 1994.
[11] C. Notredame, D. G. Higgins, and J. Heringa, "T-COFFEE: A novel method for fast and accurate multiple sequence alignment," *J. Molecular Biol.*, vol. 302, no. 1, pp. 205–217, 2000.
[12] D. J. Russell, H. H. Otu, and K. Sayood, "Grammar-based distance in progressive multiple sequence alignment," *BMC Bioinf.*, vol. 9, no. 1, p. 306, 2008.
[13] T. Lassmann, O. Frings, and E. L. Sonnhammer, "KALIGN2: High-performance multiple alignment of protein and nucleotide sequences allowing external features," *Nucleic Acids Res.*, vol. 37, no. 3, pp. 858–865, 2009.
[14] R. C. Edgar, "MUSCLE: A multiple sequence alignment method with reduced time and space complexity," *BMC Bioinf.*, vol. 5, no. 1, p. 113, 2004.
[15] K. Katoh and D. M. Standley, "MAFFT multiple sequence alignment software version 7: Improvements in performance and usability," *Molecular Biol. Evol.*, vol. 30, no. 4, pp. 772–780, 2013.
[16] O. Gotoh, "Significant improvement in accuracy of multiple protein sequence alignments by iterative refinement as assessed by reference to structural alignments," *J. Molecular Biol.*, vol. 264, no. 4, pp. 823–838, 1996.
[17] S. Needleman and C. Wunsch, "A general method applicable to the search for similarities in the amino acid sequence of two proteins," *J. Molecular Biol.*, vol. 48, no. 3, pp. 443–453, 1970.
[18] J. Pei and N. V. Grishin, "PROMALS: Towards accurate multiple sequence alignments of distantly related proteins," *Bioinf.*, vol. 23, no. 7, pp. 802–808, 2007.
[19] J. S. Papadopoulos and R. Agarwala, "COBALT: Constraint-based alignment tool for multiple protein sequences," *Bioinformatics*, vol. 23, no. 9, pp. 1073–1079, 2007.
[20] D. N. Ken and Y. Pan, "A knowledge-based multiple-sequence alignment algorithm," *IEEE/ACM Trans. Comput. Biol. Bioinf.*, vol. 10, no. 4, pp. 884–896, Jul./Aug. 2013.
[21] H. Carrillo and D. Lipman, "The multiple sequence alignment problem in biology," *SIAM J. Appl. Math.*, vol. 48, no. 5, pp. 1073–1082, 1988.
[22] T. Smith and M. Waterman, "Identification of common molecular subsequences," *J. Molecular Biol.*, vol. 147, no. 1, pp. 195–197, 1981.
[23] J. D. Thompson, T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins, "The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools," *Nucleic Acids Res.*, vol. 25, no. 24, pp. 4876–4882, 1997.

[24] K. Katoh, D. M. Standley, "MAFFT multiple sequence alignment software version 7: Improvements in performance and usability[J]," Molecular biology and evolution, vol. 30, no. 4, pp. 772–780, 2013.
[25] A. L. Rockwood, D. K. Crockett, J. R. Oliphant, and K. S. Elenitoba-Johnson, "Sequence alignment by cross-correlation," *J. Biomolecular Tech.*, vol. 16, no. 4, pp. 453–458, Dec. 2005.
[26] K. Katoh, K. Misawa, K.-i. Kuma, and T. Miyata, "MAFFT: A novel method for rapid multiple sequence alignment based on fast fourier transform," *Nucleic Acids Res.*, vol. 30, no. 14, pp. 3059–3066, 2002.
[27] T. Miyata, S. Miyazawa, and T. Yasunaga, "Two types of amino acid substitutions in protein evolution," *J. Molecular Evol.*, vol. 12, no. 3, pp. 219–236, 1979.
[28] M. Kimura, *The Neutral Theory of Molecular Evolution*. Cambridge, U.K.: Cambridge Univ. Press, 1984.
[29] R. Grantham, "Amino acid difference formula to help explain protein evolution," *Science*, vol. 185, no. 4154, pp. 862–864, Sep. 1974.
[30] S. Yamada, O. Gotoh, and H. Yamana, "Improvement in accuracy of multiple sequence alignment using novel group-to-group sequence alignment algorithm with piecewise linear gap cost," *BMC Bioinf.*, vol. 7, no. 524, pp. 1471–2105, 2006.
[31] J. D. Thompson, F. Plewniak, J.-C. Thierry, and O. Poch, "Dbclustal: Rapid and reliable global multiple alignments of protein sequences detected by database searches," *Nucleic Acids Res.*, vol. 28, no. 15, pp. 2919–2926, 2000.
[32] B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling, *Numerical Recipes in C: The Art of Scientific Computing (Fortran Version)*, 2nd ed. Cambridge, UK: Cambridge Univ. Press, 1995.
[33] M. Vetterli and H. J. Nussbaumer, "Simple FFT and DCT algorithms with reduced number of operations," *Signal Process.*, vol. 6, no. 4, pp. 267–278, 1984.
[34] H. Sorensen, M. Heideman, and C. Burrus, "On computing the split-radix FFT," *IEEE Trans. Acoust., Speech, Signal Process.*, vol. 34, no. 1, pp. 152–156, Feb. 1986.
[35] P. Duhamel and H. Hollmann, "Split-radix FFT algorithm," *Electron. Lett.*, vol. 20, pp. 14–16, Jan. 1984.
[36] P. Duhamel and M. Vetterli, "Fast Fourier transforms: A tutorial review and a state of the art," *Signal Process.*, vol. 19, no. 4, pp. 259–299, 1990.
[37] K. J. Bowers, R. A. Lippert, R. O. Dror, and D. E. Shaw, "Improved twiddle access for fast Fourier transforms," *IEEE Trans. Signal Process.*, vol. 58, no. 3, pp. 1122–1130, Mar. 2010.
[38] Y. Jiang, T. Zhou, Y. Tang, and Y. Wang, "Twiddle-factor-based FFT algorithm with reduced memory access," *presented at the Int. Parallel Distributed Processing Symp.*, Ft. Lauderdale, FL, USA, 2001.
[39] M. Frigo, C. Leiserson, H. Prokop, and S. Ramachandran, "Cache-oblivious algorithms," in *Proc. 40th Annu. Symp. Foundations Comput. Sci.*, 1999, pp. 285–297.
[40] J. Stoye, D. Evers, and F. Meyer, "Generating benchmarks for multiple sequence alignments and phylogenetic reconstructions," in *Proc. 5th Int. Conf. Intell. Syst. Molecular Biol.*, 1997, pp. 303–306.
[41] J. Stoye, D. Evers, and F. Meyer, "ROSE: Generating sequence families." *Bioinf.*, vol. 14, no. 2, pp. 157–163, 1998.
[42] S. Vinga and J. Almeida1, "Alignment-free sequence comparison-a review," *Bioinf.*, vol. 19, no. 4, pp. 513–523, 2003.
[43] R. R. Julie, D. Thompson, P. Koehl and O. Poch, "BAliBASE 3.0: Latest developments of the multiple sequence alignment benchmark," *Proteins: Struct., Function, Bioinf.*, vol. 61, pp. 127–136, 2005.

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