

# COMPUTATIONAL MOLECULAR BIOLOGY

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Hello! These lecture notes were originally created during the 2018 iteration of CS 181 (Computational Molecular Biology) taught at Brown University by Sorin Istrail. The original notes were provided by Shivam Nadimpalli, and future updates have been made by course TAs including Elliot Youth, Daniel Ben-Isvy, Sam Maffa, and Iris Huang. Please email any comments or typos to [cs1810tas@lists.brown.edu](mailto:cs1810tas@lists.brown.edu).

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(Sept. 17)

# 1 Sequence Alignment

In this chapter of the course, we will take a look at those algorithms that revolutionized the field of computational biology, and laid down robust foundations for its future.

We will first look at the problem of global alignment, after which we will consider the statistical foundations of this topic, and then finally we'll study local and gap alignment. The algorithmic technique of dynamic programming will play a key role throughout. Let's get started.

## 1.1 Global Alignment

Consider two DNA sequences  $X = \text{ATTACG}$  and  $Y = \text{ATATCG}$ . What's the optimal way to *align* these two sequences?

We haven't formally defined what any of this means, but going off our intuition, we would guess that the fewer mismatches an alignment has, the better it is. It's easy to see that the best alignment for these two sequences, then, is:

```

ATTACG
|||||
A-TATCG

```

The problem of *global alignment* of two sequences is that of finding an alignment for every residue in each sequence.

Some foreshadowing: we will soon be looking at *local* alignment, in which we look for the best alignment of any subsequences of the given sequences. The motivation behind this, as well as how it differs from global alignment, will be made clear then.

### 1.1.1 Formal setup

However, what if a (T, -) alignment is really bad for us for some reason? Or if we want to minimize the number of (A, C) alignments? We can do so by penalizing such undesirable alignments, and this can be done by choosing an appropriate scoring scheme.

Given two strings over a finite alphabet  $\Sigma$ , a *scoring scheme* is a function  $\delta : \Sigma \times \Sigma \rightarrow \mathbb{R}$ . In the case of DNA sequences,  $\Sigma = \{\text{A, T, G, C}\}$ , and in the case of protein sequences  $\Sigma$  would be the set of 20 amino acids.

It's usually convenient to represent a scoring scheme in the form of a table. For example:

	A	T	C	G
A	1	0	0	0
T	0	1	0	0
C	0	0	1	0
G	0	0	0	1

**Figure 1.** The Unitary or Diagonal Scoring Scheme.

We also introduce a related concept: the *gap penalty* is a function  $\delta : \Sigma \rightarrow \mathbb{R}$  which tells you the cost for aligning a character with a gap (-). Gaps are also called *indels* by biologists, which stands for “insertion/deletion mutations”.

**Abuse of Notation:** We will use  $\delta$  to refer to the scoring scheme and the gap penalty.

Finally, suppose we have an alignment  $x_1 \dots x_n$  and  $y_1 \dots y_n$  where  $x_i, y_i \in \Sigma \cup \{-\}$ , then the *score* of the alignment is given by  $\sum_{i=1}^m \delta(x_i, y_i)$ .

For example, under the unitary scoring scheme (Figure 1) with 0 gap penalty, the alignment of  $X$  and  $Y$  given in §1.1:

A	T	T	A	-	C	G	
A	-	T	A	T	C	G	
1	+	0	+	1	+	1	= 5

has a score of 5.

If we change the gap penalty to -1, then the score of the same alignment changes to 3. From this, it's clear that you can get different scores for the same alignment under different scoring schemes, and that the optimal alignment of two sequences depends on the scoring scheme being used.

Finally, we can formally state the problem of global alignment:

The optimal global alignment of two sequences under a chosen scoring scheme and gap penalty is the alignment of the two sequences with maximum score.

Now, how would one find this optimal global alignment? One way is to enumerate all possible alignments of the given sequences, and pick the one with the best score. But we'll see in the homework that this process is computationally inefficient. The principle of *dynamic programming*, however, comes to our rescue, as we will see in §1.1.3.

### 1.1.2 The edit graph

Let  $\Sigma$  be a finite alphabet, and let  $X = x_1 \dots x_m$  and  $Y = y_1 \dots y_n$  be strings over  $\Sigma$ . To  $X$  and  $Y$ , we associate a directed graph termed the *edit graph* of  $X$  and  $Y$ .

The vertices of the graph are the vertices of the rectangular grid of size  $(m+1) \times (n+1)$ . By  $v(i, j)$  we will mean the vertex in the grid corresponding to the  $(i+1)^{\text{th}}$  row and the  $(j+1)^{\text{th}}$  column.

Between the vertices, we have three kinds of edges:

1. A *gap-in-Y* edge: for  $1 \leq i \leq m, 1 \leq j \leq n$ , we have an edge  $v(i-1, j) \rightarrow v(i, j)$ . This edge corresponds to  $(x_i, -)$  in the alignment.
2. A *gap-in-X* edge: for  $1 \leq i \leq m, 1 \leq j \leq n$ , we have an edge  $v(i, j-1) \rightarrow v(i, j)$ . This edge corresponds to  $(-, y_j)$  in the alignment.
3. An *alignment* edge: for  $1 \leq i \leq m, 1 \leq j \leq n$ , we have an edge  $v(i-1, j-1) \rightarrow v(i, j)$ . This edge corresponds to  $(x_i, y_j)$  in the alignment.

What exactly does this graph tell us? This will become clear through the following example:

**Example 1.1.1.** Suppose we want to construct an edit graph for two sequences AC and AGC. We set up a grid of dimensions  $3 \times 4$  as shown in the picture below:



**Figure 2.** Edit Graph for AC and AGC.

Let's try looking at a path in this graph. Say we have the following sequence of vertices:

$$v(0,0) \rightarrow v(1,1) \rightarrow v(1,2) \rightarrow v(2,3)$$

Note that it can be interpreted as the following alignment:

A-C  
AGC

Similarly, suppose we're given an alignment:

AC--  
A-GC

We can construct a directed path in the graph:

$$v(0,0) \rightarrow v(1,1) \rightarrow v(2,1) \rightarrow v(2,2) \rightarrow v(2,3)$$

We come to the crucial insight which will allow us to develop an algorithm for global alignment:

Given two sequences  $X$  and  $Y$ , there is a 1-to-1 correspondence between directed paths from  $v(0,0)$  to  $v(m,n)$  in their edit graph and their alignments.

We will explore this idea further in the next section.

### (Sept. 20) 1.1.3 The Needleman-Wunsch algorithm

Recall that any path through the edit graph represents a sequence of matches, mismatches, and indels that uniquely represents one possible alignment of the two sequences. It's worth noting that in grid corresponding to the edit graph, we index from 0.

The fundamental concept of Needleman and Wunsch was that to calculate the optimal alignment score, one would only need to calculate and enumerate all the ways that an aligned pair can be added to a shorter segment to produce an alignment of an additional position in the sequences. If one always chooses the highest possible score for the extension, the highest score accumulated in the end is the global optimal score. And the path taken during the summation of the optimal score is the optimal alignment.

#### 1.1.4 The main algorithm

Suppose we have two sequences  $X, Y$  over  $\Sigma$  of lengths  $m$  and  $n$  respectively. By  $X_i$  we will represent the  $i^{\text{th}}$  character of  $X$ .

We initialize a table of size  $S$  of dimensions  $(m+1) \times (n+1)$ . By  $S_{i,j}$  we will represent the element in the  $(i, j)^{\text{th}}$  cell of our table  $S$ .

```

1: function GLOBAL ALIGNMENT( $X \in \Sigma^m, Y \in \Sigma^n$ )
2:    $S_{0,0} \leftarrow 0$ 
3:   for  $i \in \{1, 2, \dots, m\}$  do
4:      $S_{i,0} \leftarrow S_{i-1,0} + \delta(X_i, -)$ 
5:   end for
6:   for  $j \in \{1, 2, \dots, n\}$  do
7:      $S_{0,j} \leftarrow S_{0,j-1} + \delta(-, Y_j)$ 
8:     for  $i \in \{1, 2, \dots, m\}$  do
9:        $S_{i,j} \leftarrow \max \begin{cases} S_{i-1,j-1} + \delta(X_i, Y_j) \\ S_{i-1,j} + \delta(X_i, -) \\ S_{i,j-1} + \delta(-, Y_j) \end{cases}$ 
10:    end for
11:  end for
12:  return  $S_{m,n}$ 
13: end function

```

#### 1.1.5 How to obtain the optimal alignment from this table?

Obviously for many applications, we don't just want to know what the score of the optimal alignment of two sequences is, but also what the alignment that achieves this score is.

There are several strategies for obtaining the optimal alignment:

- One solution is to fill out the table in the algorithm above, and then starting at the bottom right cell of the matrix, and recalculate the max that lead to the cell's value. This time, rather than just taking the max, record the cell from which the max originated (resolving ties arbitrarily: optimal alignments aren't unique in general).

In other words, we determine whether the term of the recurrence originating from an  $X$ -gap, a  $Y$ -gap, or a match/mismatch was optimal, and backtrace to the corresponding position.

- An alternative (and computationally more efficient) solution is to place backpointers as we're computing a cell entry, indicating which cell it came from. And then we can follow these backpointers from  $S_{m,n}$  to  $S_{0,0}$  to obtain the optimal alignment.

### 1.1.6 Complexity of Needleman-Wunsch

We first look at the time complexity of the above algorithm. Indeed, we have:

- Line 2 requires 1 operation.
- Line 4 requires 3 operations, and is repeated  $m$  times.
- Line 7 requires 3 operations, and is repeated  $n$  times.
- Line 9 requires 9 operations, and is repeated  $mn$  times.
- Line 12 is a single operation.

Thus, Needleman-Wunsch runs in  $\mathcal{O}(1 + 3m + 3n + 9mn + 1) = \mathcal{O}(mn)$  time. If  $m \sim n$ , then the runtime is  $\mathcal{O}(n^2)$ , and so this is a *quadratic* algorithm.

Note that it also requires  $\mathcal{O}(mn)$  space, which is the size of our table.<sup>1</sup>

### 1.1.7 BLOSUM Matrices

As stated in the introduction in §1.2, we want to account for the probability of finding two different amino acids together during sequence alignment using empirical data. In order to do so, we use the technique of *substitution matrices* to assign match and mismatch scores more meaningfully.

The most popular type of substitution matrices are the BLOSUM (BLOcks SUBstitution Matrices) matrices introduced by Henikoff and Henikoff (1992), so named because they are created from data in the BLOCKS database. Instead of a formal construction, we'll look at a concrete example.

Let us align strings over the alphabet  $\Sigma = \{A, B, C\}$ . A *block* is just a fixed region in a given set of aligned sequences. For example, suppose that from some species whose proteins encoded over  $\Sigma$ , we obtain six sequences of the same protein that is 4 residues long from 6 different organisms. This gives us a block as follows:

Organism 1	B	A	B	A
Organism 2	A	A	A	C
Organism 3	A	A	C	C
Organism 4	A	A	B	A
Organism 5	A	A	C	C
Organism 6	A	A	B	C

**Figure 3.** Sample block.

We want to figure out what evolutionary information the block above encodes, so as to improve on our scoring scheme for sequence alignment.

Now, in our block above, we have a total of  $6 \times 4 = 24$  residues. Of these 24 residues, we have 14 As, 4 Bs, and 6 Cs.

<sup>1</sup>However, it is possible to reduce the space usage to  $\mathcal{O}(m + n)$  using Hirschberg's divide-and-conquer algorithm.

As we have 6 letters per column, there's  $\binom{6}{2} = 15$  ways of picking a pair of letters in each column. As there are 4 columns, we have  $15 \times 4 = 60$  possible ways of picking a pair of letters at the same position in the strings (i.e. in the same column) from the above table.

The table below lists the frequencies of these possible alignment pairs that we observe in the table above:

Aligned Pair	Observed Frequency
A to A	26/60
A to B	8/60
A to C	10/60
B to B	3/60
B to C	6/60
C to C	7/60

Here's an example of how the entries in the table above were computed: note that the only "B to B" alignments in the table above are in the third index in our sequences, and we have three such instances. One is between Organism 1 and Organism 4, the second is between Organism 1 and Organism 6, and the third one is between Organism 4 and Organism 6.

Now that we've gotten some observed data, let's see how it lines up with our expected data. As we have A occurring in our sequences with probability  $14/24$ , B with probability  $4/24$ , and C with probability  $6/24$  (see 2 paragraphs before table above), we should have an "A to A" alignment with probability  $14/24 \times 14/24$ .

Similarly, we must have an "A to B" alignment with probability  $2 \times 14/24 \times 4/24$  and so on. We can thus obtain expected frequencies.

We'll use the log-likelihood ratio  $f$  of each possible aligned pair in order to compare these two frequencies. This value is defined as:

$$f = 2 \times \log_2 \left( \frac{\text{Observed frequency}}{\text{Expected frequency}} \right)$$

Let's put everything together:

Aligned Pair	Observed frequency	Expected Frequency	$f$
A to A	26/60	196/576	0.70
A to B	8/60	112/576	-1.09
A to C	10/60	168/576	-1.61
B to B	3/60	16/576	1.70
B to C	6/60	48/576	0.53
C to C	7/60	36/576	1.80

**Figure 4.** BLOSUM matrix scores.

How does this help us in our alignment algorithm in any way? Indeed, we can use a scoring scheme taking the value of  $f$  in the table above, and this is a statistically-justified scoring scheme.

For example, if the observed frequency of a substitution between A and B is less than the expected frequency, then  $\delta(A, B) < 0$  as seen above. Namely, we penalize for an A-B alignment, and we can do so because from our observed data, this alignment doesn't really happen that frequently.



## 1.2 Local Alignment

Recall that in global alignment, we want to find an alignment for *every* residue in all the sequences given. What if, however, we're given two sequences that we know to be very dissimilar, but which contain some regions of similarity?

*Motivating Example 1.* Here's a silly example. Say we have two sequences TTCCCGGGAA and AAAAAAACC CGGGTTTTTT, and say we penalize mismatches with  $-2$ , gaps with  $-1$ , and alignments with  $+1$ . The optimal global alignment, then, is:

```

AAAAAA__CCCGG__TTTTTT
-----TTCCCGGAA-----

```

But what if we just want to find the region of similarity, namely CCCGGG in both sequences?

*Motivating Example 2.* Here's some more motivation rooted in biology: suppose we had 2 distantly related genomes, and we wanted to identify genes that were highly conserved between the two. In this case, we can't expect the strings to align well globally, but if we can find two regions that are highly conserved between the strings, then we can expect these regions to align well.

This is the problem of local alignment, which we will define formally in the next section.

### 1.2.1 Formal setup

Given a sequence  $X = x_1, \dots, x_m$ , a *subsequence* of  $X$  is any string of the form  $x_i x_{i+1} \dots x_j$  for some  $1 \leq i, j \leq m$ . Note that we do not preclude  $i = j$ .

Let's define the problem of local alignment.

Given  $X$  and  $Y$  over some finite alphabet  $\Sigma$ , and we want to find two subsequences  $\alpha$  and  $\beta$  of  $X$  and  $Y$  respectively, such that the global alignment score between any pair of subsequences of  $X$  and  $Y$  is maximized by  $\alpha$  and  $\beta$ .

A quick sanity check: the problem defined above allows us to identify regions of two strings that align well, even when the remainder of the strings aligns poorly. This does indeed match up with what we wanted to do, as described in our motivating examples at the start of this section.

Naively aligning all possible pairs of subsequences is prohibitively expensive from a computational viewpoint. In the next section, we'll see how to make slight changes to our algorithm for global alignment to get an efficient algorithm for local alignment.

### 1.2.2 The Smith-Waterman Algorithm

Given two sequences  $X$  and  $Y$  of length  $m$  and  $n$  respectively, we set up the edit graph and dynamic programming table  $S$  for  $X$  and  $Y$  just as we did for global alignment. We also carry over the notation used in that section.

Here's the algorithm for local alignment:

```

1: function LOCAL ALIGNMENT( $X \in \Sigma^m, Y \in \Sigma^n$ )
2:    $S_{0,0} \leftarrow 0$ 
3:   for  $i \in \{0, 1, 2, \dots, m\}$  do
4:      $S_{i,0} \leftarrow 0$ 
5:   end for
6:   for  $j \in \{1, 2, \dots, n\}$  do
7:      $S_{0,j} \leftarrow 0$ 
8:   end for
9:   for  $j \in \{1, 2, \dots, n\}$  do
10:    for  $i \in \{1, 2, \dots, m\}$  do
11:       $S_{i,j} \leftarrow \max \begin{cases} 0 \\ S_{i-1,j-1} + \delta(X_i, Y_j) \\ S_{i-1,j} + \delta(X_i, -) \\ S_{i,j-1} + \delta(-, Y_j) \end{cases}$ 
12:    end for
13:  end for
14:  return  $\max\{S_{i,j} \mid 0 \leq i \leq m, 0 \leq j \leq n\}$ 
15: end function

```

How does backtracking work in this case? It's the same as in the global case, except we start backtracking from  $\max\{S_{i,j} \mid 0 \leq i \leq m, 0 \leq j \leq n\}$ , i.e. the cell in the table with the maximum value (as opposed to from  $S_{m,n}$  as in the case of global alignment), and we stop as soon as we encounter a cell with entry 0.

### 1.2.3 How's this different from global alignment?

Let  $S$  be the table for our alignment algorithm as above. In the case of global alignment, a table entry  $S_{i,j}$  corresponded to the optimal alignment of the  $i^{\text{th}}$ -prefix of  $X$  (denoted by  $X[i]$ ) and the  $j^{\text{th}}$ -prefix of  $Y$  (denoted by  $Y[j]$ ).

What does an arbitrary table element correspond to in the case of local alignment? If you think about it,  $S_{i,j}$  corresponds to the optimal global alignment between all the suffixes of all the prefixes of  $X[i]$  and  $Y[j]$ .

Finally, in the case of global alignment, adding a constant value to all entries of the scoring matrix does not alter the optimal alignment (this only changes the scores of the alignments, but the *relative* scores remain the same). This, however, isn't the case with local alignment. Note that there are no negative numbers in the local alignment table.

### 1.2.4 Complexity of Smith-Waterman

In the algorithm above, lines 3 and 6 require a single operation each, the first being executed  $m + 1$  times and the second  $n$  times. In line 10, we maximize over 5 cases, requiring an additional comparison relative to the global alignment for a total of 10 operations, and we execute this line  $nm$  times. In line 13, we maximize over  $nm$  items. Let's say each item requires a load and a comparison operation, for a total of  $2nm$  operations.

The total number of operations is then  $m + n + 12nm + 1$  operations, which again is in  $\mathcal{O}(nm)$ .

(Sept. 20) **1.3 Alignment with Gaps**

We'll next look at a alignment with a special emphasis on gaps.

**1.3.1 Some biological motivation**

When a protein is synthesized, the DNA is *transcribed* from DNA to RNA. RNA is a similar macromolecule to DNA: the sugar deoxyribose is replaced by ribose, and the Thymine base (T) is replaced by Uracil (U).

As we will see in the HW, DNA consists of large chunks of non-coding DNA called *introns*. These introns, once the DNA is transcribed into RNA, are removed via a process called *splicing*. The remaining sequences, called *exons*, are *translated* into protein.

Now, biologists can usually obtain this RNA from cells, but unfortunately it's already undergone splicing, i.e. the non-coding regions have been removed. Using this RNA, we can obtain DNA complementary to it, which is called cDNA.

In order to figure out what proteins parts of DNA are coding for, we want to find the location of this cDNA in the genome. Namely, we wish to align this cDNA within the genome. But this isn't as easy as it appears on first sight...

(Sept. 27) **1.3.2 Gap Penalties**

Recall that we wanted to align the cDNA of a protein transcript to the nuclear DNA from which it was transcribed. The difficulty arises in that large sections of nuclear DNA are excised via splicing before we get the mature RNA, so we will need to be tolerant of large gaps in our alignment. However, we don't want there to be too many of these gaps, and we don't want them to be too small.

In this case, we have a somewhat different notion of *gap*. A small gap and a big gap are in some sense equivalent, as we don't care how *long* the introns are, rather we want to work under the assumption that there aren't "too many" of them.

One way to approach this situation is to pick an appropriate scheme to penalize gaps during sequence alignment. We do so by means of *gap functions*.

Formally, a *gap function* is a map  $w(l) : \mathbb{Z} \rightarrow \mathbb{R}$ . Here,  $l$  is an integer representing the length of a gap, and  $w(l)$  is the amount by which we penalize a gap of length  $l$ . A few different gap functions of varying degrees of usage are presented in the table below:

Gap Function	$w(l)$
Linear	$\tau l$
Affine	$\gamma + \tau l$
Logarithmic	$\gamma + \tau \log(l)$
Quadratic	$\gamma + \tau l^2$

You can think of  $\gamma$  as the "opening penalty", and  $\tau$  as the "extension penalty".

Note that the linear gap function is the usual gap penalty that we used in the earlier sections. Relative to the linear gap function, affine and logarithmic gap penalties favor long gaps, whereas the quadratic gap penalty allows for short gaps but penalizes longer gaps. Make sure your intuition for these functions matches their mathematical form.

### 1.3.3 Global alignment with gap function

In this subsection, we'll look at how adding a gap function to our scoring scheme alters our global alignment algorithm.

Adding a gap function requires us to use 4 matrices to keep track of the optimal alignment—think about why this is the case. We set them up as follows:

$$V_{i,j} = \max(E_{i,j}, F_{i,j}, G_{i,j})$$

$$G_{i,j} = V_{i-1,j-1} + \delta(x_i, y_j)$$

$$E_{i,j} = \max_{k \in \{0,1,\dots,i-1\}} V_{i,k} - w(j-k)$$

$$F_{i,j} = \max_{l \in \{0,1,\dots,j-1\}} V_{l,j} - w(i-l)$$

Here  $V_{i,j}$  gives the gapped alignment cost of prefixes of lengths  $i, j$ .  $V$  is defined in terms of  $G$ ,  $E$ , and  $F$ .  $G_{i,j}$  gives the optimal gapped alignment score of prefixes of  $x, y$  of lengths  $i, j$ , conditional on the fact that the  $i$ th and  $j$ th characters are *aligned*.  $E$  and  $F$  on the other hand give optimal alignments of prefixes conditional on the last character containing a gap:  $E_{i,j}$  is conditional on the fact that the terminal position in the alignment contains a gap in sequence  $x$ , and  $F_{i,j}$  requires that the alignment ends on a gap in sequence  $y$ .

With these definitions, clearly  $V_{i,j}$  is correct: note that  $E_{i,j}$ ,  $F_{i,j}$ , and  $G_{i,j}$  give the optimal gapped alignment cost under three mutually exclusive conditions, and  $V_{i,j}$  gives the optimal amongst these.  $G_{i,j}$  requires that the final character pair are aligned, so its score is identical to the *aligned* (no gap) case in linear gapped alignment.  $E_{i,j}$  represents the case where  $x$  has a gap. We consider all possible alignments that end in an  $x$  gap: such an alignment can start with an arbitrary amount of the  $y$  string consumed. The maximum over  $k$  represents the maximum score over alignments of the prefix of length  $k$  of  $y$  and length  $i$  of  $x$ , and then the remainder of  $x$  is filled with gaps in the alignment. Similarly, we may flip  $x$  and  $y$  to make the same argument for  $F_{i,j}$ .

### 1.3.4 Complexity

Using this recurrence, we can handle *any* gap penalty function. However, we pay an asymptotically significant cost in terms of time, and a constant factor cost in terms of memory, to use this technique. Because the maxima in  $E$  and  $F$  are over a non-constant number of cells, the time complexity increases. Each such maximum needs to examine no more than  $\max(m, n)$  cells of  $V$ , and there are  $2mn$  cells in  $E$  and  $F$ .  $G$  on the other hand only needs to examine a constant number of cells, so evaluating it is efficient, requiring  $\mathcal{O}(mn)$  time, and by the same reasoning, evaluating  $V$  also requires  $\mathcal{O}(mn)$  time. The total time to evaluate every cell in each of these four matrices is thus  $\mathcal{O}(mn) + \mathcal{O}(mn) + \mathcal{O}(mn(m+n)) + \mathcal{O}(mn(m+n)) = \mathcal{O}(mn(m+n))$ , asymptotically<sup>2</sup>.

<sup>2</sup>Note that  $\mathcal{O}(\max(m, n)) = \mathcal{O}(m+n)$ : to see this, consider the following:  $\lim_{m,n \rightarrow \infty} \frac{\max(m, n)}{m+n} \leq \frac{\max(m, n)}{2 \max(m, n)} = \frac{1}{2}$ .

Note also that the memory requirement increases. Rather than requiring  $(m+1)(n+1)$  matrix cells, we now require  $4(m+1)(n+1)$  matrix cells. However, this change is only by a constant factor, so there is no asymptotic difference.