Getting statistical significance and Bayesian confidence limits for your hidden Markov model or score-maximizing dynamic programming algorithm,

with pairwise alignment of sequences as an example

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GCGAA--CGACGTCAGGCAGA---TCTAGA
CCGAAGCCGA-GCCGGG--AAGCGTGTTGA
```

$m = 25, n = 27$

You can do #1, but want to do #2 and #3:

Example: Sequence Alignment

1. For two sequences, of lengths m and n, what is the optimal alignment A and what is its score S?
2. Is S statistically significant given m and n? — is it unlikely to arise with random sequences?
3. Is A credible? — are other plausible alignments of these sequences substantially the same?

You can do #1, but want to do #2 and #3:

Example: Word Wrapping Text

1. For a paragraph of words, what is the optimal way to divide them into lines A, and how pretty is it S?
   E.g., $S = -\sum w_i^2$, where $w_i =$ spaces added to line i
2. Is S unusual? — Is this paragraph of words particularly hard (or easy) to wrap?
3. Is A special? — are other reasonable word wrappings of these words similar?

You can do #1, but want to do #2 and #3:

Problem Statement

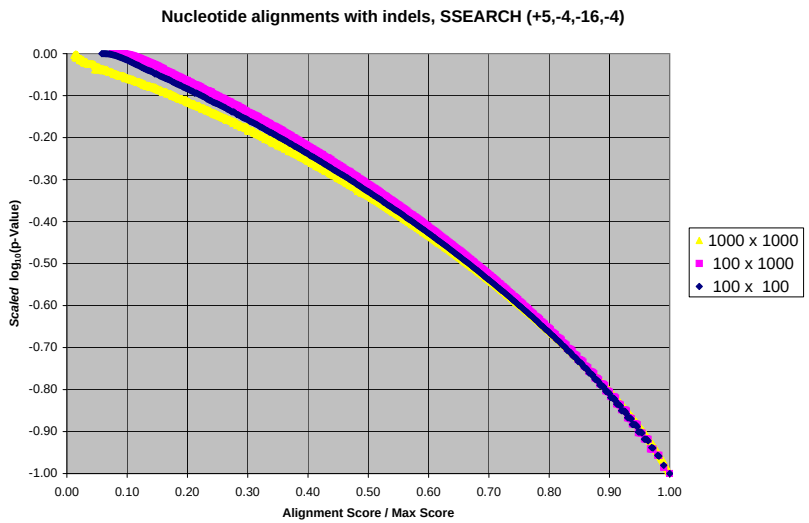
1. Optimization: Find and evaluate an optimum using a dynamic programming algorithm, hidden Markov model, or partition function calculation.
2. Hypothesis Testing: What is the probability that random inputs would score as well? Null distribution. *p*-value.
3. Bayesian Confidence Limits (a.k.a. Credibility Limits):
   - What fraction of solution space has exactly d differences from the optimum, for $d = 0, \ldots, d_{\max}$. Difference distribution.
   - How many differences must be allowed to capture 95% of solution space? 95% credibility limit.

Note: Uncertainty of individual features (*e.g.*, a specific alignment match) is valuable, but not our goal.

Results: Statistical Significance vs. Score

For Smith & Waterman (1981) sequence alignment, score and statistical significance are related, but . . .

- relationship is non-trivial and depends upon input size.



Protein-protein alignment

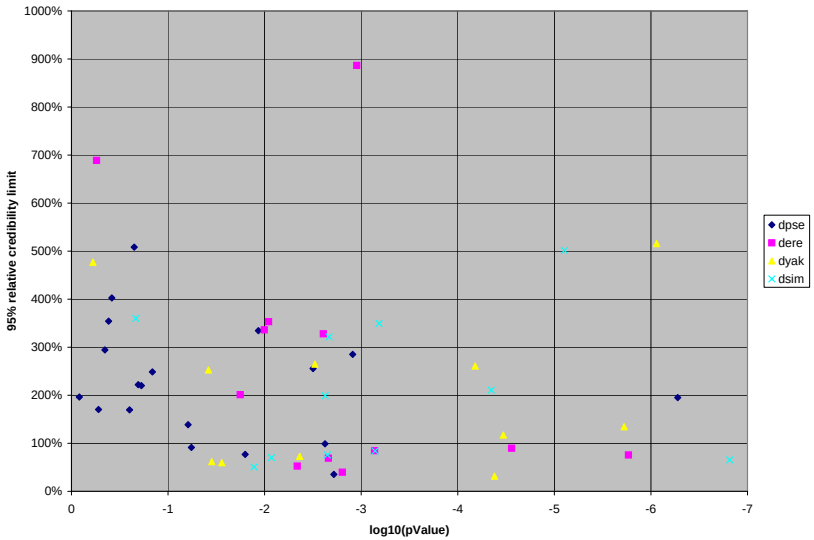
Nucleotide-nucleotide alignment

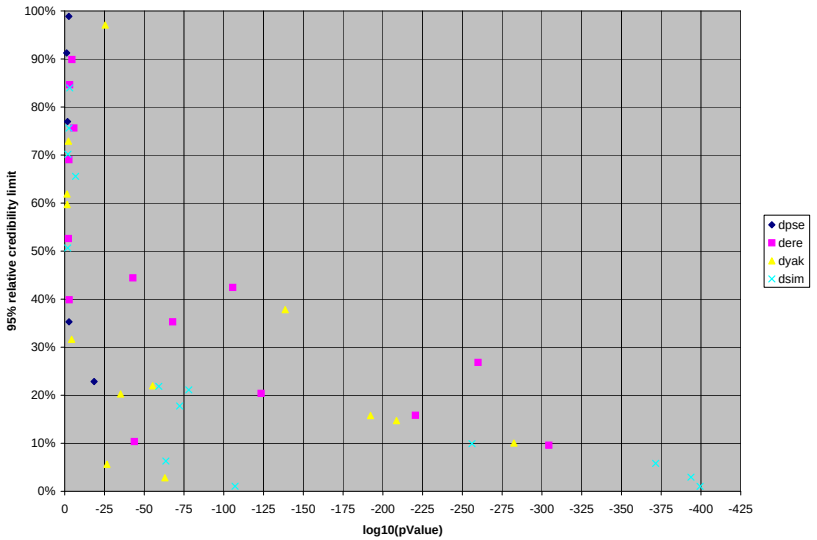
Compare: Karlin & Altschul (1990)

Results: Credibility vs. Statistical Significance

Significance and Bayesian confidence are related, but . . .

- poor credibility exists even at superb *p*-values.

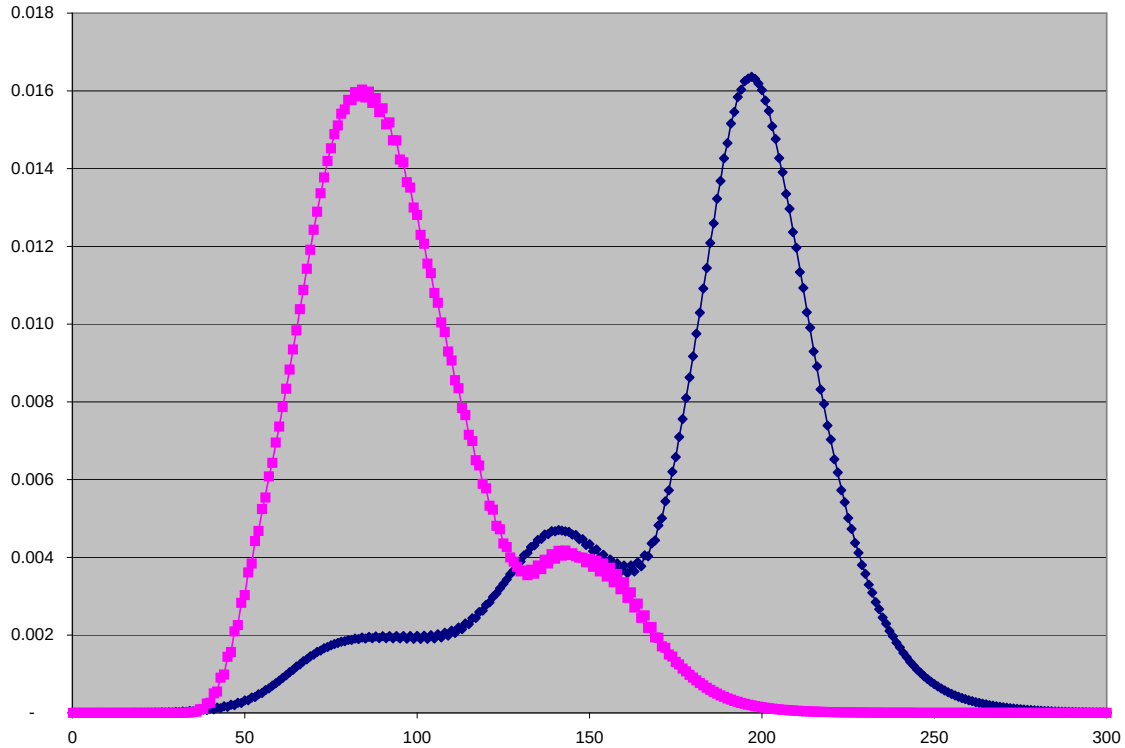




20 gene promoters of *Drosophila melanogaster* aligned to orthologous regions in four other fly genomes.

Results: Distribution of Differences

For Smith-Waterman sequence alignment, the distribution of differences can have a rich structure.



Orthologous Human (1677 nt) vs. Mouse (1666 nt).
Viterbi(Dark) = 450 bp, Centroid(Light) = 438 bp.

Algorithms for Discrete High-Dimensional Inference

Many problems are tackled with dynamic programming:

Hidden Markov Model

- Sequence alignment: HMMER
- Protein folding: HMMSTR / ROSETTA

Partition Function Computation / Markov Random Field

- RNA secondary structure: Sfold

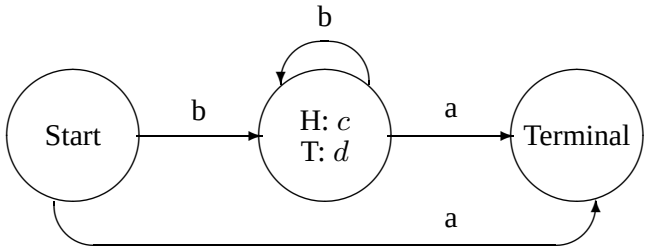
Viterbi / Maximum Score / Minimum Energy

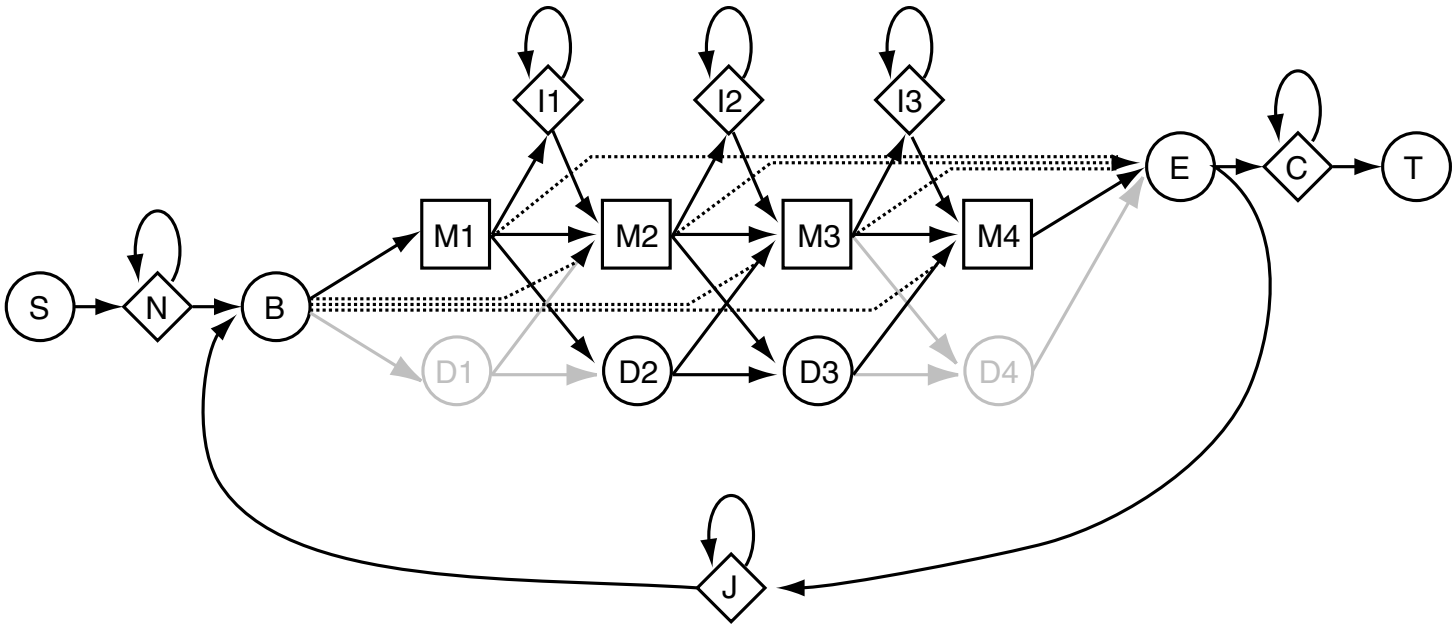
- Seq. Alignment: Smith-Waterman, Needleman-Wunch
- RNA secondary structure: Mfold

Collectively, *Hidden Boltzmann Models*

Hidden Boltzmann Models

Flipping a biased coin





A Plan7 Profile-HMM (Eddy, 2003)

Also: Viterbi vs. Forward, Smith & Johnson (2007)

Estimating Statistical Significance

Naïve Sampling

1. Generate some random examples from the null.
2. Observe the fraction that score as well as your result.

Need $\mathcal{O}(1/p)$ samples for a small p-value. ☹

Importance Sampling

Similar to simulated annealing.

0. Establish a probability model, if absent.
1. Choose a temperature.
2. Generate random samples at the new temperature.
3. Compute temperature-corrected fraction $\geq$ your result.

Need 100–10,000 samples, even for $p = 10^{-4000}$. ☺

Newberg (2008, 2009)

0. Establish a Probability Model

An *emission path* through the computation has a . . .

Dynamic programming algorithm: score, computed by addition of encountered transition and emission scores.

HMM (or Partition function): (unnormalized) probability or odds ratio, computed by multiplication.

Convert a Dynamic Programming Algorithm To Multiplications

- For each score s, instead use an unnormalized probability

$$Z = \exp(\lambda s) \; .$$

  E.g., $\lambda = \ln(10)/5$ gives $Z \mapsto 10Z$ when $s \mapsto s + 5$.
- Addition of scores $\rightarrow$ multiplication of Zs.
- Maximum of scores $\rightarrow$ addition of Zs.

1. Choose a Temperature

Use a reasonable *ad hoc* procedure to obtain T.

- Generally, want 20-60% of instances $\geq$ your result.

2. Generate Samples

Goal: Instead of from the null, generate input instances from a temperature-biased distribution.
E.g., generate a pair of sequences (x, y) for alignment.

Watch out: two pages of math headed our way!

2. Generate Samples

1. Use HMM *forward algorithm* to sum over paths, but
   - Use $Z^{1/T}$ in lieu of each Z.
   - Also sum out emissions d for each emitter e using

$$\left\langle Z_e^{1/T} \right\rangle = \sum_d Z_e(d)^{1/T} \Pr_{\text{null}}(d) \ .$$

2. Use HMM *backtrace* to sample a path, but
   - Also sample each emission d with probability

$$\frac{Z_e(d)^{1/T} \Pr_{\text{null}}(d)}{\left\langle Z_e^{1/T} \right\rangle} \ .$$

   - Discard the sampled transitions.

Result: An input instance, with bias for higher scores.

3. Compute Temperature-Corrected Fraction

Naïve Sampling: For significance of result Z_0 (or p_0 or s_0)

$$p(Z_0) = \sum_{\text{all } (x,y)} \Pr_{\text{null}}(x,y)\Theta(Z(x,y) \geq Z_0) \ ,$$

where $\Theta(\text{true}) = 1$ and $\Theta(\text{false}) = 0$.

Importance Sampling

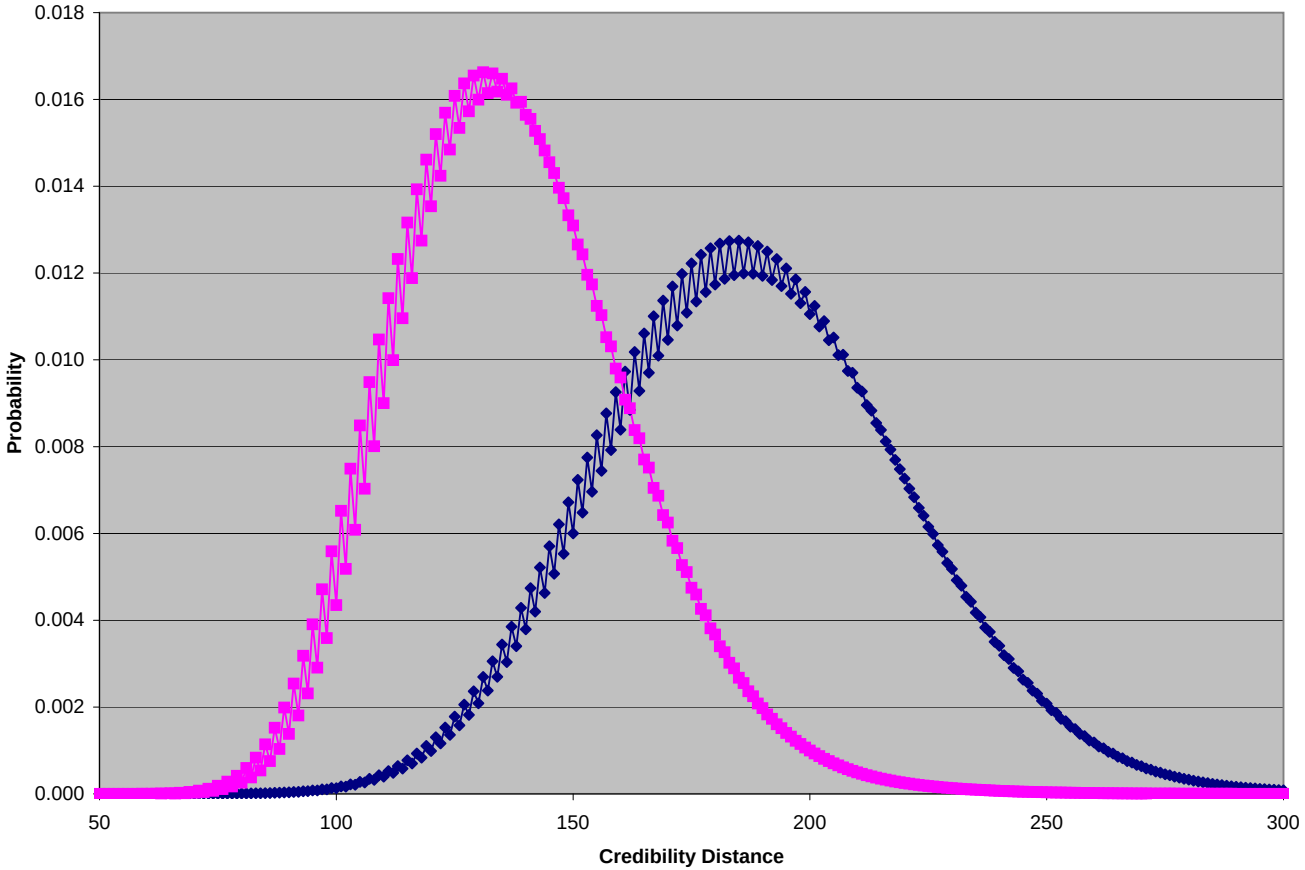
$$p(Z_0) = \sum_{\text{all } (x,y)} \Pr_T(x,y) \frac{\Pr_{\text{null}}(x,y)\Theta(Z(x,y) \geq Z_0)}{\Pr_T(x,y)}$$

$$\widehat{p(Z_0)} = \frac{1}{N} \sum_{(x,y)\sim \Pr_T} \frac{\Pr_{\text{null}}(x,y)\Theta(Z(x,y) \geq Z_0)}{\Pr_T(x,y)}$$

Done with statistical significance!

Computing Bayesian Confidence Limits

How do we efficiently compute this (or its cumulative form)?



Newberg & Lawrence (2009)

Bayesian Confidence Limits

0. Establish a probability model, if absent.
1. Choose an integer difference measure.

Use *Sampling Approach* (Webb-Robertson *et al.*, 2008), *Direct Approach*, *Polynomial Approach*, or

Fourier Transform Approach

2. Choose a integer (with only small factors) that is a little larger than the maximum number of differences.
3. Run modified *forward algorithm* to compute each Fourier transform coefficient (in parallel).
4. Fourier transform the coefficients.

Direct Approach

Unaltered Sequence Alignment Algorithm (Simplified)

Algorithm's typical step looks something like:

$$\begin{aligned} Z[i,j] = \; & Z[i-1,j-1]\,Z_M(x_i,y_j) + \\ & Z[i-1,j]\,Z_D(x_i) + \\ & Z[i,j-1]\,Z_I(y_j) \end{aligned}$$

Goal is $Z[m,n]$, where m and n are input strings' lengths.

Direct Approach

Recap: Unaltered Algorithm

$$\begin{aligned} Z[i,j] = \; & Z[i-1,j-1]\,Z_M(x_i,y_j) + \\ & Z[i-1,j]\,Z_D(x_i) + \\ & Z[i,j-1]\,Z_I(y_j) \end{aligned}$$

Difference Distribution via the Direct Approach

Number of ways to get differences d. Typical step:

$$\begin{aligned} Z[i,j,d] = \; & Z[i-1,j-1,d-\Delta_M(i,j)]\,Z_M(x_i,y_j) + \\ & Z[i-1,j,d-\Delta_D(i)]\,Z_D(x_i) + \\ & Z[i,j-1,d-\Delta_I(j)]\,Z_I(y_j)\ , \end{aligned}$$

where Δ is the number of new differences.
Goal is $Z[m,n,d]$ for all possible total differences d.
Requires increased runtime and memory. ☹

Polynomial Approach

Recap — Difference Distribution via the Direct Approach:
$Z[m,n,d]$ is number of ways to get score d.

$$\begin{aligned} Z[i,j,d] = \; & Z[i-1,j-1,d-\Delta_M(i,j)]\, Z_M(x_i,y_j) + \\ & Z[i-1,j,d-\Delta_D(i)]\, Z_D(x_i) + \\ & Z[i,j-1,d-\Delta_I(j)]\, Z_I(y_j)\ . \end{aligned}$$

Difference Distribution via the Polynomial Approach

$P[i,j]$ is a polynomial in indeterminant ω that "packs" the $Z[i,j,d]$ values. Define $P[i,j] = \sum_d Z[i,j,d]\omega^d$. Typical step:

$$\begin{aligned} P[i,j] = \; & P[i-1,j-1]\, Z_M(x_i,y_j)\, \omega^{\Delta_M(i,j)} + \\ & P[i-1,j]\, Z_D(x_i)\, \omega^{\Delta_D(i)} + \\ & P[i,j-1]\, Z_I(y_j)\, \omega^{\Delta_I(j)}\ . \end{aligned}$$

Seeking $P[m,n]$ polynomial.
Still increased runtime and memory. ☹

Fourier Transform Approach

Recap — Difference Distribution via the Polynomial Approach: $P[m,n]$ is a polynomial that packs the difference distribution.

$$\begin{aligned} P[i,j] = \; & P[i-1,j-1]\, Z_M(x_i,y_j)\, \omega^{\Delta_M(i,j)} + \\ & P[i-1,j]\, Z_D(x_i)\, \omega^{\Delta_D(i)} + \\ & P[i,j-1]\, Z_I(y_j)\, \omega^{\Delta_I(j)} \; . \end{aligned}$$

Difference Distribution via the Fourier Transform Approach

Can recover coefficients of $P[m,n]$ with via its valuation at sufficiently many points. Its value for a fixed ω is from:

$$\begin{aligned} C[i,j] = \; & C[i-1,j-1]\, Z_M(x_i,y_j)\, \omega^{\Delta_M(i,j)} + \\ & C[i-1,j]\, Z_D(x_i)\, \omega^{\Delta_D(i)} + \\ & C[i,j-1]\, Z_I(y_j)\, \omega^{\Delta_I(j)} \; . \end{aligned}$$

Coefficients recovery is efficient via Discrete Fourier Transform, so let $\{\omega_0,\ldots,\omega_{r-1}\}$ be the rth complex roots of unity.

function ComputeScoreDistribution
for $k \in \{0, \ldots, r-1\}$
    $\omega = \cos(2\pi k/r) + i \sin(2\pi k/r)$
    $f(k) =$ BackgroundExec(CalcFourier(ω))
WaitForBackgroundProcesses
return DiscreteFourierTransform(f)

function CalcFourier(ComplexNumber ω)
for $i \in \{0, \ldots, m\}$
    for $j \in \{0, \ldots, n\}$
        $C[i,j] = C[i-1,j-1]\, Z_M(x_i, y_j)\, \omega^{\Delta_M(x_i,y_j)} + C[i-1,j]\, Z_D(x_i)\, \omega^{\Delta_D(x_i)}$
            $+ C[i,j-1]\, Z_I(y_j)\, \omega^{\Delta_I(y_j)}$
return $C[m,n]$

- Serial algorithm has original memory requirement. ☺
- Parallel algorithm has (nearly) original runtime. ☺

Concluding Observations

For dynamic programming algorithms, hidden Markov models, and partition function calculations

- . . . optimum score, statistical significance (p-value), and credibility / Bayesian confidence limits are not fungible.

Solutions

In many cases, if you can optimize score then you can

- . . . estimate even a very extreme p-value.
- . . . calculate the difference distribution and credibility limits.

Links `http://www.rpi.edu/~newbel/publications/`

Statistical Significance of sequence alignments: Newberg (2008)
Statistical Significance of hidden Boltzmann models: Newberg (2009)

Credibility: Newberg & Lawrence (2009)