UFO: a tool for unifying biomedical ontology-based semantic similarity calculation, enrichment analysis and visualization
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Supplementary File 1

Semantic Similarity Measures

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Biomedical ontologies are represented in a directed acyclic graph (DAG). In this section, we introduce eleven implemented between-term semantic similarity measures (including eight node-based, two edge-based and one hybrid-based) and eleven between-entity measures (including four pairwise-based and seven group-wise-based ones). First, we introduce information content (IC) of a term. Then, we define semantic similarity measures between terms and between entities.

1. INFORMATION CONTENT

The IC of a term is calculated based on a corpus, (*i.e.*, an annotation database of HPO (Köhler, et al., 2014)) as following:

\[ IC_t = -\log(p_t) \]

where \( p_t \) is the probability of a term occurred in a given corpus (Lord, et al., 2003):

\[ p_t = \frac{f_t}{t_{\text{root}}} \]

where

\[ f_t = \text{Annot}_t + \sum_{c \in \text{Children}_t} f_c \]

where \( \text{Annot}_t \) is number of proteins annotated with term \( t \) in a corpus and \( \text{Children}_t \) is the set of children of term \( t \) in given ontology graph.

Figure 1 illustrates Information Content (IC) calculation for Gene Ontology (GO) term using Gene Ontology and Annotation databases.

**Figure S1**: Illustration of Information Content (IC) Calculation for Gene Ontology term using Gene Ontology and Annotation databases.

In the UFO tool, we visualize IC of terms by color. Figure 2 visualizes ICs of some HPO terms by their color (the higher IC of the term is the redder of node is)
II. BETWEEN-TERM MEASURES

The ontology data is represented in directed acyclic graph, in which each term is located in a node and the relations between terms are represented by edge connecting nodes. Therefore, calculation for semantic similarity between terms are categorized into two main methods: i) node-based and ii) edge-based.

A. Node-based measures

In this section, we firstly introduce four node-based measures for similarity between terms. They are all based on most informative common ancestors (MICA) of the terms, which is defined as following:

$$IC_{MICA} = \max_{c \in SP(t_1, t_2)} (IC_c)$$

where $SP(t_1, t_2)$ are shared ancestors of terms $t_1$ and $t_2$.

Figure 3 shows shared ancestors and MICA term of two HPO terms (HP:0000008 and HP:0000010)
First, Resnik (Resnik, 1995) defined the similarity between two terms as following:

$$ IC_t = -\log(p_t) $$

$$ SimT_{Resnik}(t_1, t_2) = IC_{MICA} $$

Second, other between-term similarity measures proposed by (Lin, 1998), (Jiang and Conrath, 1997) and (Schlicker, et al., 2006) were defined respectively as following:

$$ SimT_{Lin}(t_1, t_2) = \frac{2 \times IC_{MICA}}{IC_{t_1} + IC_{t_2}} $$

$$ SimT_{JIC}(t_1, t_2) = \frac{1}{IC_{t_1} + IC_{t_2} - 2 \times IC_{MICA} + 1} $$

$$ SimT_{Ret}(t_1, t_2) = \frac{2 \times IC_{MICA} \times (1 - P_{MICA})}{IC_{t_1} + IC_{t_2}} $$

where $$ P_{MICA} = 10^{-IC_{MICA}} $$

Similarly to the four between-term similarity measures, (Couto, et al., 2005) defined common disjunctive ancestors (CDA) of terms $$ t_1 $$ and $$ t_2 $$ to replace MICA in the four measures as following:

$$ IC_{CDA} = \frac{\sum_{t \in CDA} IC_t}{|CDA|} $$

where CDA contain common disjunctive ancestors of terms $$ t_1 $$ and $$ t_2 $$

Figure 4 shows CDA terms (HP:0000118 and HP:0000119) of two HPO terms (HP:0000008 and HP:0000010)
Therefore, four more between-term similarity measures were defined: Sim\textsubscript{T\textsubscript{ResnikGrasM}}, Sim\textsubscript{T\textsubscript{LinGrasM}}, Sim\textsubscript{T\textsubscript{JGrasM}} and Sim\textsubscript{T\textsubscript{RelGrasM}}. In summary, a total of eight node-based between-term similarity measures were used in our study.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure_s4}
\caption{Shared/Common ancestors: Shared ancestors (HP:0000001, HP:0000118 and HP:0000119) and CDA terms (HP:0000118 and HP:0000119) of two HPO terms (HP:0000008 and HP:0000010)}
\end{figure}

\textbf{B. Edge-based measures}

In this section, we introduced two widely used edge-based between-term semantic similarity measures. The first one is simply defined as length of longest path from lowest common ancestor (LCA) to root, Sim\textsubscript{T\textsubscript{Wu}}(t\textsubscript{1}, t\textsubscript{2}) = (LCA, root) (Wu, et al., 2005). Meanwhile, the second one (Yu, et al., 2005) additionally takes consider on length of longest path between each term to LCA and formally defined as following:

\[
\text{Sim}_{Yu}(t_1, t_2) = \frac{L(\text{LCA}, \text{root})}{L(\text{LCA}, \text{root}) + L(t_1, \text{LCA}) + L(t_2, \text{LCA})}
\]

where \( L(t_1, t_2) \) length of the longest path between two terms \( t_1 \) and \( t_2 \)

Figure 5 shows the longest path of two HPO terms (HP:0000008 and HP:0000010).
Figure S5: The longest path (red line) of two HPO terms (HP:0000008 and HP:0000010).

C. Hybrid-based measures

We implemented one hybrid measure, which was introduced in (Wang, et al., 2007). In the study, an ontology term \( t \) is represented as \( \text{DAG}_t = (t, T_t, E_t) \) where \( T_t \) is the set of ontology terms in \( \text{DAG}_t \), including term \( t \) and all of its ancestor terms in the ontology graph, and \( E_t \) is the set of edges connecting the ontology terms in \( \text{DAG}_t \). To quantitatively compare two ontology terms, a semantic value of term \( t \) is defined as the aggregate contribution of all terms in \( \text{DAG}_t \) to the semantics of term \( t \) as following:

\[
SV(t) = \sum_{t_i \in T_t} S_t(t_i)
\]

where

\[
S_t(t_i) = \begin{cases} 
S_t(t) = 1 & \text{if } t_i = t \\
S_t(t_i) = \max\{w_e * S_t(c) | c \in \text{children}_{t_i}\} & \text{if } t_i \neq t
\end{cases}
\]

and \( 0 < w_e < 1 \)

Finally, a semantic similarity between two terms, \( t_1 \) and \( t_2 \), is defined as follow:

\[
\text{Sim}_{Hybrid}(t_1, t_2) = \frac{\sum_{t_i \in T_{t_1} \cap T_{t_2}} (S_{t_1}(t_i) + S_{t_2}(t_i))}{SV(t_1) + SV(t_2)}
\]

Figure 6 shows DAGs of two HPO terms (HP:0000008 and HP:0000010).
Figure S6: DAGs of two HPO terms (HP:0000008 and HP:0000010).

III. BETWEEN-ENTITY MEASURES

For assessment of similarity between two annotated entities, two main approaches have been proposed, i.e., pairwise and groupwise.

Assuming that two entities $e_1$ and $e_2$ are annotated by a set of terms $T_1 = \bigcup_{k=1}^{m} t_{1k}$ and $T_2 = \bigcup_{i=1}^{n} t_{2i}$. In this section, we are going to introduce between-entity similarity measures.

A. Pairwise

This approach calculates the similarity between two entities based on the similarity of every pair terms which annotate to the entities.

First, the similarity of every pair of terms is calculated to generate a similarity matrix as following (see Fig. 7):

$$
\begin{array}{cccc}
| t_{11} & \cdots & t_{1n} | & | t_{21} & \cdots & t_{2n} | \\
| SimT(t_{11}, t_{21}) & \cdots & SimT(t_{1n}, t_{2n}) | & MaxRow_1 \\
\hline
| t_{1m} | & \cdots & | t_{2m} | \\
| SimT(t_{1m}, t_{21}) & \cdots & SimT(t_{1m}, t_{2n}) | & MaxRow_m \\
\hline
| MaxCol_1 & \cdots & MaxCol_n |
\end{array}
$$
Figure S7. Example of a semantic similarity matrix, where shadding cells are elements of the matrix, $t_{ij}$ are column and cell headers, $MaxRow_i$ and $MaxCol_i$ are maximum values of row $i$ and column $i$, respectively.

Then, four pairwise between-entity were defined as following:

The first two measures were simply either based on average (Lord, et al., 2003) or maximum (Sevilla, et al., 2005) similarity of all pairs.

$$SimE_{Avg} = \frac{\text{Avg}_{i=1...m;j=1...n} (SimT(t_{1i}, t_{2j}))}{\text{MaxCol}_i = \max_{i=1...m;j=1...n} (SimT(t_{1i}, t_{2j}))}$$

Meanwhile, Couto et al (Couto, et al., 2005) and Azuaje et al (Azuaje, et al., 2005) opted for a composite average in which only the best-matching term pairs are considered (best-match average):

$$SimE_{BMA} = \frac{\sum_{i=1...m} \text{MaxRow}_i + \sum_{j=1...n} \text{MaxCol}_j}{n_{\text{Row}} + n_{\text{Col}}}$$

Finally, Schlicker et al (Schlicker, et al., 2006) proposed a variation of the best-match average:

$$SimE_{RCMax} = \max_{i=0...m} ( \text{Avg} (\text{MaxRow}_i), \text{Avg} (\text{MaxCol}_j))$$

B. Groupwise

This approach is categorized into two main methods: i) vector-based and ii) graph-based. In which, two popular vector-based between-entity similarity measures Cosine (Huang, et al., 2007) and Kappa (Chabalier, et al., 2007) were defined based on cosine and kappa coefficients. More specifically, $T_1$ and $T_2$ were first represented as binary vectors as following:

$v_1 = (v_{11}, ..., v_{1N}), v_2 = (v_{21}, ..., v_{2N})$

$$v_{1k}, v_{2l} = \begin{cases} 1 & \text{if } t_k, t_l \in T \\ 0 & \text{otherwise} \end{cases}$$

where $T$ is whole set of $N$ terms in the ontology database. Then, the cosine of the two vectors $v_1$ and $v_2$ is calculated:

$$SimE_{\text{Cosine}}(v_1, v_2) = \frac{\sum_{i=1}^{N} (v_{1i} \times v_{2i})}{\sqrt{\sum_{i=1}^{N} (v_{1i} \times v_{1i})} \times \sqrt{\sum_{i=1}^{N} (v_{2i} \times v_{2i})}}$$

Another vector-based between-entity similarity measure is based on Kappa coefficient. Formally, it is defined as following:

$$Kappa(v_1, v_2) = \frac{p_a - p_e}{1 - p_e}$$

where:
- $P_o$ is observed proportionate agreement: $(P_{11} + P_{00})/P_t$
- $P_e$ is overall probability of random agreement: $P_{e1} + P_{e0}$
- $P_{v1=1}$ is probability $v_1=1$: $(P_{11} + P_{10})/P_t$
- $P_{v2=1}$ is probability $v_2=1$: $(P_{11} + P_{01})/P_t$
- $P_{v1=0}$ is probability $v_1=0$: $(P_{01} + P_{00})/P_t$
- $P_{v2=0}$ is probability $v_2=0$: $(P_{10} + P_{00})/P_t$
- $P_t$, $P_{11}$, $P_{00}$, $P_{10}$ and $P_{01}$ is total number of observations, number of observations where $v_1 = v_2 = 1$, number of observations where $v_1 = 1$ and $v_2 = 0$, and number of observations where $v_1 = 0$ and $v_2 = 1$, respectively.

For graph-based measures, T1 and T2 is extended with ancestors of terms in each set. Therefore, they are defined as follows:

$$T_1 = \bigcup_{k=1}^{m} (t_k \cup \text{anc}(t_k))$$
$$T_2 = \bigcup_{l=1}^{n} (t_l \cup \text{anc}(t_l))$$

Five more graph-based between-entity similarity measures were defined as following:

A measure is based on term overlap (Lee, et al., 2004)

$$SimE_{TO} = |T_1 \cap T_2|$$

A normalized version of $SimE_{TO}$ (Mistry and Pavlidis, 2008)

$$SimE_{NTO} = \frac{|T_1 \cap T_2|}{\min(|T_1|, |T_2|)}$$

A measure is based on Jaccard index (Gentleman, 2005; Martin, et al., 2004)

$$SimE_{UI} = \frac{|T_1 \cap T_2|}{|T_1 \cup T_2|}$$

An IC-based weighted version of $SimE_{UI}$ (Pesquita, et al., 2007)

$$SimE_{GIC} = \frac{\sum_{t_k \in T_1 \cap T_2} |IC_{t_k}|}{\sum_{t_l \in T_1 \cup T_2} |IC_{t_l}|}$$

Finally, a longest path-based between-entity similarity measure (Gentleman, 2005):

$$SimE_{LP} = \max_{t_k \in T_1 \cap T_2} L(t_k, \text{root})$$

In summary, a total of eleven between-entity similarity measures were used in our study.

IV. ENRICHMENT ANALYSIS

Given an entity set ($S_e$) and an ontology term $t$, let $H_0$ denotes the null hypothesis that there is no significant association between $S_e$ and $t$. The association between $S_e$ and $t$ is defined as an overlap ($k$) between the $S_e$ and the set of entities annotated with term $t$ in the corpus. There are
three statistical tests popularly used to test whether the overlap is significant or not (i.e., $H_0$ is rejected or not) (Rivals, et al., 2006), i.e., Fisher’s exact test (equivalent to Hypergeometric test), Binomial test and Chi-squared test. In UFO, we implemented the Fisher’s exact test and the Binomial test.

The above problem can be formulated as following 2x2 contingency table.

| Entities annotated with t | Entity set ($S_e$) | Non-entity set (the remaining) |
|--------------------------|-------------------|-------------------------------|
| a (k)                    | b                 | a+b (K)                       |
| c (n-k)                  | d                 | c+d (N-K)                     |
| a+c (n)                  | b+d               | N                             |

Fisher showed that the probability of obtaining the overlap (with an observed value $k$) was given by the hypergeometric distribution with parameters $N$, $n$, and $K$ (Agresti, 1992; Fisher, 1922):

$$P(x=k) = \frac{\binom{a+b}{K} \binom{c+d}{N-K}}{\binom{N}{n}}$$

where:
- $N$ is number of annotated entities in the corpus (e.g., number of genes in the corpus which are annotated with GO terms)
- $K$ is number of entities annotated with term $t$ in the corpus (e.g., number of genes in the corpus which are annotated by a specific GO term).
- $n$ is number of entities in the entity set ($S_e$) (e.g., a gene set of interest which we want to find GO terms significantly annotating to).
- $k$ is number of entities in the entity set which are annotated with term $t$.

For a large sample, the overlap has approximately a binomial distribution

$$P(x=k) = \binom{K}{k} p^k (1-p)^{n-k}$$

where:
- $p$ is success probability in the population (i.e., $K/N$, the probability that an entity is annotated with term $t$ in the corpus).
- $K$ is the number of success states in the population (i.e., number of entities annotated with term $t$ in the corpus).
- $n$ is the number of draws (i.e., number of entities in the entity set ($S_e$)).
- $k$ is the number of observed successes (i.e., number of entities in the entity set which are annotated with term $t$).

$S_e$ is said to be enriched by $t$ if there is statistically significant overlap between entity set ($S_e$) ($n$) and the set of entities annotated with term $t$ in the corpus ($K$).

When testing multiple hypotheses, the obtained $p$-values have to be corrected in order to control the type I error (false positive) rate (Noble, 2009). In UFO, we implemented two multiple testing correction methods, i.e., Bonferroni, and Benjamini and Hochberg correction. The former (i.e., Bonferroni) strongly controls the probability of making at least one type I error (i.e., the family-wise error rate (FWER)) for tests (Bonferroni, et al., 1936); meanwhile, the latter is to control the false discovery rate (FDR), i.e. the expected proportion of false positives among the positively identified tests (Benjamini YaY, 2001).
After applying a multiple testing correction method, an adjusted p-value was obtained for each ontology term $t$. The p-value represents the probability of the null hypothesis; thus, the smaller p-value is the less likely that the association between the entity set ($S_e$) and that term is random. In enrichment analysis, the p-value $\leq 0.05$ indicates the association is statistically significant.

V. SIMILARITY BETWEEN TWO ENTITY SETS

The procedure to calculate the similarity between two sets of entities is as following:

- For each set of entities, a set of ontology terms statistically significant annotating for the entity set is identified. Thus, each entity set is now equivalent to a meta-entity which is annotated with the set of significant ontology terms.
- The similarity between two entity sets is now equivalent to the similarity between two meta-entities, that can be calculated by any of between-entity similarity measures (see section III. BETWEEN-ENTITY MEASURES).
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