

Use of a Hierarchical Oligonucleotide Primer Extension Approach for Multiplexed Relative Abundance Analysis of Methanogens in Anaerobic Digestion Systems

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The anaerobic digestion process can degrade the organic substance in waste and wastewater into methane as a renewable energy source. The process is usually operated using a reactor under specific conditions to enrich trophic groups of microorganisms, with which the complex substance is hydrolyzed and fermented to the methanogenic precursors for methane formation. Owing to the key position in the terminal step, the reactor performance ultimately relies on the activities of the methanogen population. To increase the efficiency of organic matter decomposition and methane recovery, understanding of the relevant population structure and the ecological significance inside the reactor is needed. We established a multiplexed hierarchical oligonucleotide primer extension (HOPE) method to detect the relative abundances of methanogens at hierarchical specificities in anaerobic digestion systems. Totally, a set of 27 primers were designed to target the total archaeal populations and methanogens from 22 genera within 4 taxonomic orders. After optimization for their specificities and detection sensitivity under the conditions of multiple single-nucleotide primer extension reactions, the HOPE approach completed the abundance analysis of the methanogens on 19 consortium samples (i.e., 513 reactions) within 8-9 hr. Among the samples, the methanogens detected with order-level primers accounted for >77.2% of total archaeal populations. The samples typically comprised 2 to 7 known methanogen genera within orders *Methanobacteriales*, *Methanomicrobiales*, and *Methanosarcinales* and displayed population dynamic and spatial distributions in anaerobic reactor operations. Principal component analysis further showed that the sludge samples could be clustered into 3 distinctive groups, in accordance with the distribution of the acetotrophic *Methanosaeta*, hydrogenotrophic *Methanolinea*, and methylotrophic *Methanomethylovorans*, respectively. Considering its high specificity, technical simplicity, rapidness, and cost-effectiveness, as well as robust multiplexing capabilities, HOPE can be used as a platform for the routine monitoring of methanogen populations for improved management of anaerobic processes. Whenever analysis of a large number of samples is needed, HOPE can be an effective method, too.



Deciphering mycorrhizal fungi in cultivated *Phalaenopsis* microbiome with next-generation sequencing of multiple barcodes

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Traditionally, scientists depend on in vitro and in vivo culturing to explore fungal communities. Most species remain unculturable, and rare strains can be easily unexploited in culture. Technically optimizing culture conditions for individual species, especially when the species composition of a community remains unknown, is time-consuming and difficult. Next-generation sequencing, a huge-scale nucleotide-sequence-based tool, can overcome such difficulties and provide high sensitivity. Here, we deciphered the microbiome of cultivated orchid roots based on amplicon-based metagenomics with 6 fungus-specific DNA barcodes. In total, 205 genera among 64 orders of fungi were identified in healthy *Phalaenopsis* roots, 21 of which are able to form mycorrhiza with host plants. It is noticeable that 74 % of the detected genera were exclusively uncovered with single barcodes, indicating that the use of multiple barcodes was necessary. To deal with the high heterogeneity in the data and to integrate the information on species composition across barcodes, a rank-scoring strategy was developed. As a result, of the top 10 most abundant genera, 3 were mycorrhizal fungi and another 5 were beneficial to plants. Taken together, these barcodes provided complementary and comprehensive power in deciphering the microbial diversity in orchid roots, especially in revealing rare species.



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Estimation of physical functional disabilities and long-term care needs for patients under maintenance hemodialysis

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Background and objectives: Physical functional disabilities in hemodialysis (HD)

patients may increase their mortality and long-term care needs. This study aims to estimate the changes of proportion for different physical functional disabilities and the lifelong care needs along time after beginning HD.

Methods: We used a population-based cohort consisting of 84,657 incident HD patients in Taiwan between 1998 and 2009 to estimate the survival function and extrapolate to lifetime through a semi-parametric method. The Barthel Index (BI) was used to measure functional disability levels cross-sectionally in 1,334 HD patients recruited from 9 HD centers. A BI score less than 50 was considered as severe disability. Lifetime care needs were obtained by extrapolating the age-stratified survival functions to lifetime and then multiplying them with proportions of different kinds of functional disabilities over time.

Results: On average, HD patients had at least 6.4, 2.0, and 1.3 years without disability, with moderate disability, and severe disability, respectively. The most common care needs were stair-climbing and bathing which were 3.0 and 1.7 years, respectively. HD patients were expected to have about 3 years living with disabilities for those beginning HD at an age above 35; however, the older the patient, the higher the proportion of functional disabilities and care needs.

Conclusions: HD patients are in need of long-term care and require early intervention and resource planning. The method developed in this study can also be applied to other chronic illnesses with various functional disabilities.



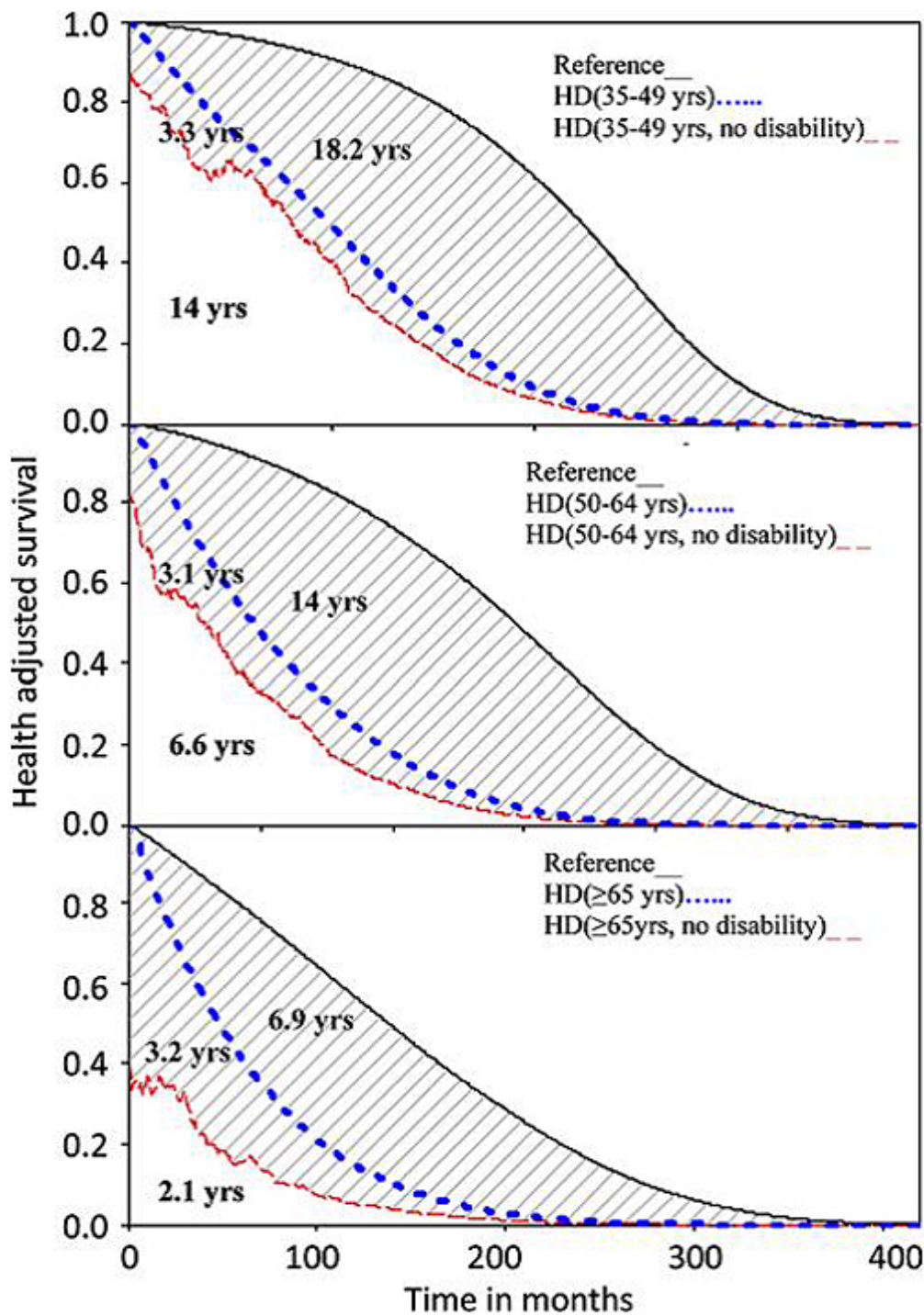


Figure 1. The lifetime health-adjusted survival of hemodialysis (HD) patients. Each panel illustrates the sum of expected years of life lost (shaded area between the solid and dotted curves) and the lifespan with functional disabilities (shaded area between the dotted and dashed curves) for HD patients stratified by age.

Two-Level Hierarchical Alignment for Semi-Coupled HMM-Based Audiovisual Emotion Recognition with Temporal Course

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A complete emotional expression typically contains a complex temporal course[1] in face-to-face natural conversation. In this study, we focused on exploring the temporal evolution of an emotional expression for audio-visual emotion recognition. A novel data fusion method with respect to the temporal course modeling scheme named Two-Level Hierarchical Alignment-Based Semi-Coupled Hidden Markov model (2H-SC-HMM)^[1] is proposed to effectively solve the problem of complex temporal structures of an emotional expression and consider the temporal relationship between audio and visual streams for increasing the performance of audio-visual emotion recognition in a conversational utterance.



Temporal Course of Emotional Expression

Previous psychologist research^{[2][3][4]} showed that a complete emotional expression can be characterized in three sequential temporal phases: Onset (application), Apex (release), and Offset (relaxation), when considering the manner and intensity of expression. Although the temporal course of emotional expression was demonstrated sequentially in time, a complete emotional expression is expressed by more than one utterance in natural conversation, and in more detail, each utterance may contain several temporal phases of emotional expression as shown in Fig. 1.

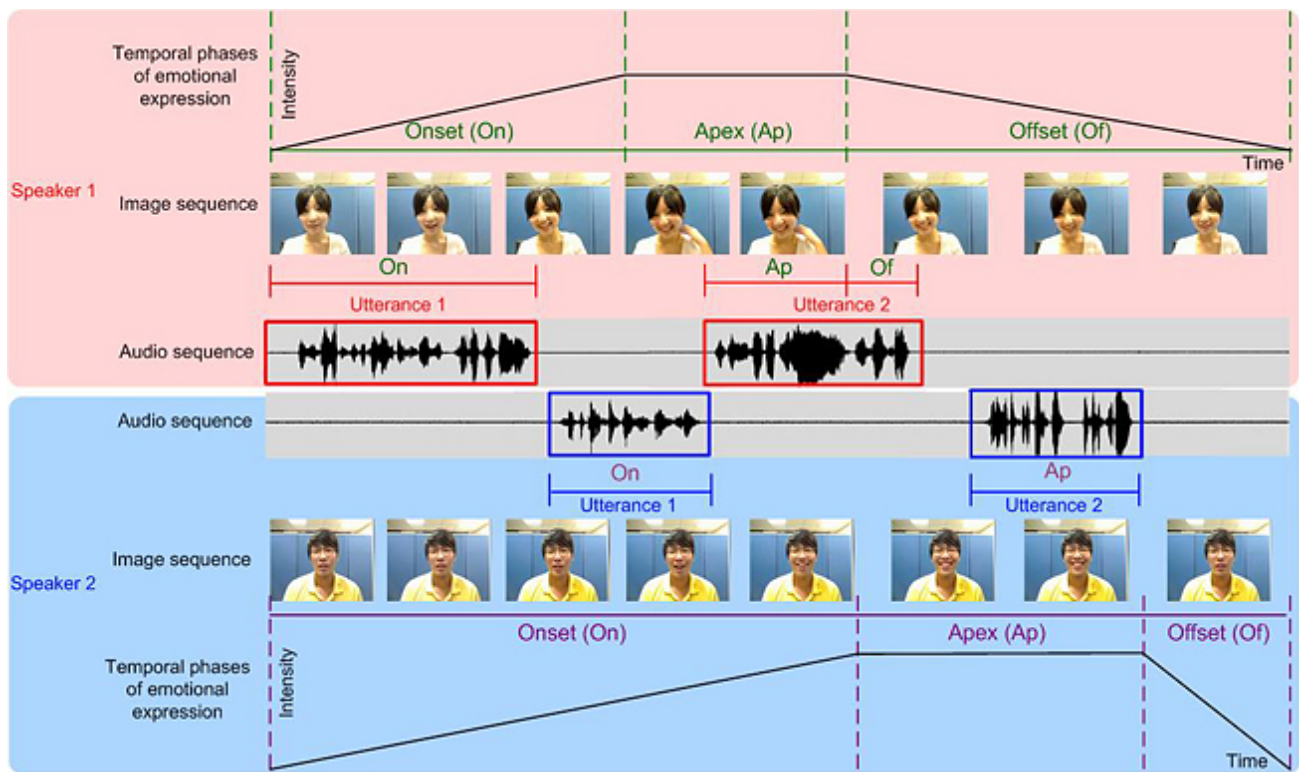


Figure 1. An example of various temporal phases of happy emotional expression occurred to different utterances in a real conversational environment.

Modeling the Temporal Course of Emotional Expression

Based on aforementioned analysis, to model the complex temporal course of emotional expression, a temporal course modeling scheme is proposed in this study to characterize the temporal evolution involved in an emotional state that occurs in an isolated utterance. An isolated utterance in a conversation can express one or several emotional sub-states, which are defined to represent the temporal phases (i.e., onset, apex, or offset with low or high intensity) of an emotional expression, and an HMM is used to characterize single emotional sub-state, rather than the entire emotional state as shown in Fig. 2.

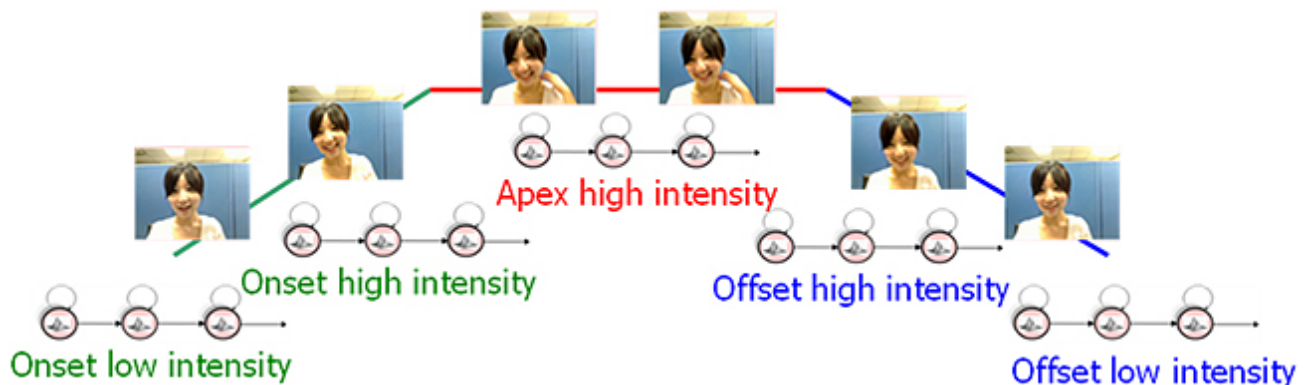


Figure 2. Emotional Temporal phases modeling based on various HMMs

Two-Level Hierarchical Alignment-Based Semi-Coupled Hidden Markov Model (2H-SC-HMM)

For effective emotion recognition, a two-level hierarchical alignment mechanism is further proposed and applied to the SC-HMM to align the relationship within and between the temporal phases in the audio and visual HMM

sequences at the model and state levels as shown in Fig. 3. By integrating the emotional sub-state language model, which model the temporal transition between emotional sub-states expressed in an isolated utterance, the proposed two-level hierarchical alignment-based SC-HMM (2H-SC-HMM) can further provide a constraint on allowable temporal structures to obtain an optimal recognition result of emotional state in each utterance. The formula of the proposed 2H-SC-HMM is shown as

$$\hat{E} = \arg \max_E \left\{ \max_{\Lambda^a, \Lambda^v} \left[\underbrace{P(\Lambda^v | \Lambda^a, E)}_{\text{隱藏式馬可夫模型序列辨識機率}} \underbrace{P(\Lambda^a | E)}_{\text{隱藏式馬可夫模型序列狀態序列校準機率}} \max_{S^a, S^v} \left(\underbrace{P(O^a, S^a | \Lambda^a, E)}_{\text{隱藏式馬可夫模型序列校準機率}} \underbrace{P(S^v | S^a, \Lambda^a, E)}_{\text{情緒子狀態語言模型}} \right) \underbrace{P(O^v, S^v | \Lambda^v, E)}_{\text{隱藏式馬可夫模型序列校準機率}} \underbrace{P(S^a | S^v, \Lambda^v, E)}_{\text{情緒子狀態語言模型}} \right] \underbrace{P(E)}_{\text{模型序列機率}} \right\}$$

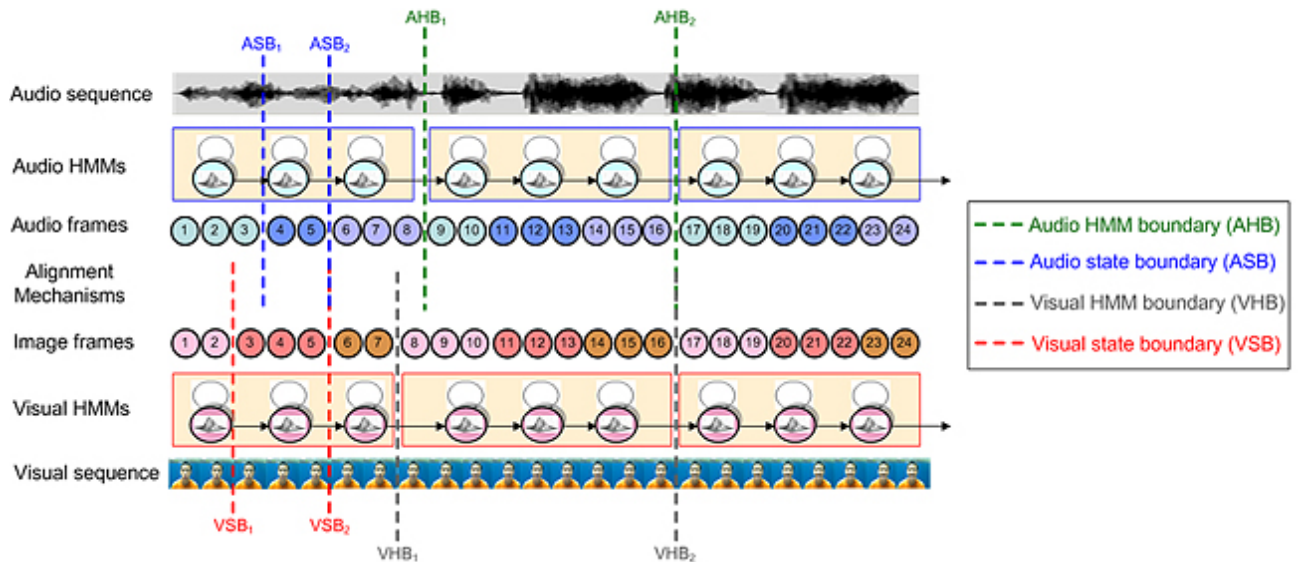


Figure 3. An example illustrating model- and state-level alignment between audio and visual HMM sequences in the happy emotional state. The green and gray dotted lines represent the audio and visual HMM boundaries respectively and are used for model-level alignment estimation; the blue and red dotted lines represent the state boundaries under audio and visual HMMs respectively and are used for state-level alignment estimation. The audio and image frames are represented by the numbered circles.

For performance evaluation, two databases^{[5][6][7]} are considered: the posed MHMC database and the spontaneous SEMAINE database. The recognition accuracy achieved 91.55% and 87.5% for posed MHMC database and the spontaneous SEMAINE database, respectively. Experimental results show that the proposed method not only outperforms other fusion-based bimodal emotion recognition methods for posed expressions but also provides acceptable results for spontaneous expressions.

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