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There are many objects and substances around us, like dyes and plants, which contain both the reflective and fluorescent components. To separate the reflective component from the fluorescent component is helpful in examining the intrinsic status of objects. Therefore, effective methods for this separation task are needed. Existing methods for fluorescence and reflectance separation needs multiple shots under different illuminations, thus are inapplicable to dynamic scenes. This research first uses a single hyperspectral image, and designs the optimal illumination spectrum for this separation task. The fact that existing hyperspectral cameras are still not very fast in data capture leads us to explore the possibility of reconstructing hyperspectral images from RGB images. On the application aspect, a simple separation method of weak fluorescence in the presence of strong environmental illumination has been developed and successfully used for freshness examination of meat and cheese.

1.研究開始当初の背景

(1) Fluorescence is a common optical phenomenon present in many natural objects, like corals, sea fishes and minerals. Fluorescent substances have also been widely applied to paper and cloth for color augmentation, to biochemical dyes for molecular labeling and tracking, as well as to currency notes for anti-counterfeiting. To examine the fluorescent spectrum and the spatial distribution of fluorescence is of great importance in object classification, origin identification, food freshness examination, and so on. On the one hand, a fluorescent object would reflect back the incident lighting at the same wavelength, just acting like a typical reflective object. On the other hand, it would absorb some lighting at shorter wavelengths, and emit it at longer wavelengths. Therefore, the observed image of a fluorescent scene is composed of the reflective component and the fluorescent component. Therefore, it is necessary to develop effective methods to separate the fluorescent and reflective component.

(2) Existing separation methods in both spectral and RGB domain need several shots under different illuminations. This arouses the necessity of exchanging illuminations in sequence and the operation of illumination and camera synchronization. Such methods are inappropriate to dynamic scenes.

2.研究の目的

(1) The first research objective is to develop an effective method to separate the fluorescent and reflective components by using a single hyperspectral image.

(2) The second objective is to reconstruct hyperspectral images from three-channel RGB images, so as to avoid the high cost of a video-rate hyperspectral camera.

(3) The third objective is to separate weak fluorescence in the presence of strong environment illumination, for the purpose of food freshness examination by examining the distribution of fluorescence emitting substances.

3.研究の方法

(1) For the objective 2-1, we first notice the high correlation of the fluorescent spectra and the reflective spectra by using public datasets. The immediate outcome is that, using an ordinary illuminant, like an LED lamp, these two components are ambiguous. Therefore, we algorithmically design the optimal illumination spectrum to disambiguate these two components, and produce optimal filters to realize such illumination. The physical restrictions in producing filters should be incorporated into the numerical design process.

(2) For the objective 2-2, we first investigate the spectral distribution of fluorescent and reflective objects in existing public datasets. The dimension of the spectral space is usually quite low, which allows to reconstruct hyperspectral images from multispectral images.

(3) For the objective 2-3, the reflective component is overwhelmingly strong in the presence of strong environmental illumination, yet the emitted fluorescent light is quite weak, especially for bacteria lying on the food surface. To handle this extremely challenging case, we allow two shots so as to cancel out the environment illumination. The shading effect due to the surface geometry needs to be eliminated as well, for the sake of easy visibility of fluorescent emitting substances.

4.研究成果

(1) High correlation between the reflective and fluorescent emission spectra. We first noticed that these two components are highly correlated, that is, the bases of the fluorescent spectra can actually represent the reflective spectra, and vice versa. This implies that when the illumination spectrum is flat, these two components cannot be separated at all. Many existing lamps for daily use, like LED and incandescent tube, have relatively flat spectra, thus they are inappropriate for the separation task.

Figure 1. Separation using LED and Fluo. Lamp.

Figure 1 shows the separation results of using an LED lamp and a fluorescent lamp, from which we can see that the separation using the spiky fluorescent illumination spectrum is much better than using the smooth LED illumination spectrum.

(2) Now, the natural question is how the optimal illumination spectrum looks like and how to find/realize it. Since the imaging model is linear in terms of the reflective spectrum and the fluorescent spectrum, we represent the two components by using bases, and model the illumination spectrum design problem into a mathematical optimization of condition number minimization. To realize the illumination spectrum physically, there are several constraints that should be enforced in the optimization process. The illumination spectrum is an element-wise multiplication of the known wideband illumination (for example, the ideal flat white illumination) and the transmittance curve of the filter. So, the transmittance curve should be nonnegative. Due to technical limitations of existing filter production techniques, there is an upper bound on the reproducible transmittance curve, and very shape details cannot be reproduced. By considering this restriction in mind, we represent the transmittance curve as the linear combination of cosine basis curves with varying frequency. We add the nonnegative constraints on the linear combination and limit the maximum allowed frequency.

Figure 2 shows the designed optimal illumination spectra with the nonnegative constraint only and with maximum frequency restriction. We can observe that the designed illumination with nonnegative constraints are very spiky, which is hard to produce using a single filter. On the contrary, the designed illumination spectrum with both the nonnegative constraints and the maximum frequency restriction is relatively smooth, thus can be realized in a single film filter.

(3) Application to live coral fluorescence analysis. Our developed single-shot separation method of the fluorescent and reflective components has been used to examine live coral in Okinawa. Coral exists fluorescence due to the fluorescent protein. It remains unknown to biologists whether it is possible to identify the fluorescent protein by observing the fluorescent emission only. Our method has attracted attention from domestic coral researchers, and it has been successfully applied to separate the fluorescence of coral as shown in Figure 3.

Figure 3. Fluorescence separation of live coral.

(4) Spectral image reconstruction from RGB. Existing hyperspectral cameras to capture dynamic scenes are very expensive, yet RGB cameras are quite cheap. This has inspired us to explore the feasibility of reconstructing a hyperspectral image from its RGB version. We first use nonlinear dimensionality reduction to analyze the existing dataset of reflective scenes. We recognize that the dimensionality of natural scenes is very low, thus it is feasible to recover spectra from RGB observations. A shallow neural network is used to map an RGB coordinate to a point in the hidden space, and a sparse dictionary based method is developed to reconstruct spectra from the hidden space. The experiment shows that the proposed method outperforms the state-of-the-art methods in terms accuracy and stability. One example of the reconstructed images at several bands and the error images is shown in Figure 4.

Error Images (Hotmap Color Coded)

Figure 4. Sample bands of a reconstructed hyhyperspectral image from RGB. The error images are coded in color. Blue denotes small error.

(5) Extension to fluorescent and reflective scenes. We have explored the feasibility of using the above method onto scenes with fluorescence. The dimensionality of scenes with fluorescence is higher, so larger number of bands is needed. In spite of that, we have approximately recovered hyperspectral fluorescent scenes from RGB images. Figure 5 shows one example of food samples with fluorescence in the shrimp region. It can be observed from the error image that the reconstructed image is very close to the ground truth.

Reconstructed Images Error Image Figure 5. Recovered scenes with fluorescence.

(6) Weak fluorescence visualization for food freshness examination. When food goes bad, some fluorescent emitting bacteria will grow on the food surface. Therefore, it is possible examine the freshness of food by detecting the fluorescence distribution. However, these fluorescence emitting bacteria absorb deep UV light, which is uncommon in daily illuminants. Therefore, the fluorescent emission of deteriorating food is usually very weak when observed in ordinary indoor/outdoor environment. This is especially true when the environment illumination is strong, which causes strong surface reflectance. For this challenging case, we use two RGB images. The first one is captured under the UV light, and the second one is captured under the visible light. Both images can be captured with unknown environment illumination. We develop a linear separation method for separation and cancel out the shading effect as well, which is caused by the surface geometry. This method has been successfully used to visualize the bacteria distribution of deteriorating pork and cheese over time, as shown in Figure 6.

 Tuna Figure 6. Visualize the distribution of fluorescence emitting bacteria for freshness examination by separating fluorescence from the reflectance component.

(7) The fluorescence and reflectance separation method has aroused strong interest from a giant domestic maker of fluorescence imaging devices for biomedical research. The separation method is now being properly adapted, so as to match their product. Our method is expected to contribute to an evolutional improvement in their product, that is, the old single-point imaging will be replaced by our image-plane area imaging. The details will be omitted because of business considerations.

5.主な発表論文等

(研究代表者、研究分担者及び連携研究者に は下線)

〔学会発表〕(計 2 件)

○¹ . Misaki Meguro, Yuta Asano, Yinqiang Zheng, Imari Sato, Visibility enhancement of fluorescent substance under ambient illumination using flash photography, In Proceedings of 2017 International Conference on Image Processing, pp. 1622-1626, Beijing, China, September, 2017, Oral.

(2). Yan Jia, <u>Yinqiang Zheng</u>, Lin Gu, Art Subpa-Asa, Antony Lam, Yoichi Sato, Imari Sato, From RGB to Spectrum for Natural Scenes via Manifold-Based Mapping, In Proceedings of 2017 International Conference on Computer Vision, pp. 4715-4723, Venice, Italy, October, 2017, Poster. 6.研究組織 (1)研究代表者 鄭銀強(ZHENG, Yinqiang) 国立情報学研究所・コンテンツ科学研究 系・助教 研究者番号:30756896 (2)研究分担者 (3)連携研究者 (4)研究協力者 佐藤いまり(SATO, Imari) 目黒美沙紀(MEGURO, Misaki) 浅野祐太(ASANO, Yuta) ジアヤン(JIA, Yan) グリン(GU, Lin) 佐藤洋一(SATO, Yoichi)