Deconvolution, Confocal data,
Markov Random Fields,
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EDGE-PRESERVING REGULARIZATION FOR CONFOCAL DATA DECONVOLUTION

Point spread function (PSF, also termed impulse response) is an important characteristics of imaging devices and as such it can be with advantage used for data reconstruction and restoration. In confocal microscopy the non-ideal PSF introduces significant blurring. Therefore, restoration methods based on the so called deconvolution were developed, which utilize the PSF estimated directly from the scanned data (blind deconvolution) or obtain it from other sources. A problem of the well-known Richardson-Lucy (R-L) algorithm is that it does not have to converge when no regularization is used.
In this paper, we propose a fully three-dimensional deconvolution algorithm which extends the R-L algorithm by using an edge-preserving regularization term based on data modeling by Markov random fields.

1 INTRODUCTION

The main goal of restoration techniques is to suppress image degradation exploiting knowledge of its nature. In confocal microscopy, images are corrupted by undesired contribution of out-of-focus intensities due to non-ideal point spread function (PSF) of the device. Such PSF can be estimated by observing the behavior of light originating from a point source and passing through the microscope optics. Subsequently, PSF can be used to quantitatively compensate for the blurring of images due to out-of-focus information in a process called deconvolution.

In confocal microscopy (CM) the non-ideal PSF introduces significant blurring, and moreover, in confocal laser scanning microscopy it is not only device- but also sample-dependent. Traditional image restoration techniques for confocal microscopy are in details described in Vlient *et al* [13]. Further, restoration methods based on the so-called blind deconvolution were developed [2], which estimate the PSF on a per voxel basis directly from the scanned data. This, unfortunately, leads to a large the number of estimated parameters and thus also to prohibitively large computational times. In this paper we propose a fully three-dimensional deconvolution algorithm with blind but also with traditional estimation of the PSF of the microscope. The traditional PSF estimation can be done by recording images of subresolution point sources in the form of, for example, various types of fluorescent microspheres with diameter bellow $0.2\mu m$. The algorithm issues from modeling by Markov random fields (MRF) and from maximum likelihood (ML) estimation techniques and other

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Bayesian approaches (Richardson-Lucy algorithm [10]), and extends our previous work on object reconstruction in tomographic data [16, 15].

Images reconstructed by deconvolution manifest sharper edges and details, lower background influence, better contrast, and improved signal-to-noise ratio. Such images are therefore suitable for further interactive and automated evaluation techniques, predominantly in the areas of analysis of biological structures at sub-cellular resolution. However, processing and data deconvolution is often alleviated by a huge amount of data (up to 256 images of 2048×2048 pixels, each with 32 spectral bands).

In this paper, in Section 2 we describe related work, while the topic of Section 3 is deconvolution theory. Here, the Richardson-Lucy (R-L) algorithm, maximum-aposteriori methods and regularization are introduced. Subsequently, in Section 4 we propose additional regularization methods for the R-L algorithm issuing from the MRF-based modeling. Finally, in Section 5 we present results and conclusion.

2 RELATED WORK

Classical image reconstruction techniques by linear restoration filters [5] are based on minimization of mean square (Wiener filter) or least square errors (Tikhonov-Miller). Under special conditions, the Tikhonov-Miller approach can be represented as a Wiener filter [14]. Linear image restoration has two central problems connected with (i) band-limited character of the imaging technology, and (ii) noise degradation of the images. Due to them, linear reconstruction is not appropriate for reconstruction of confocal images. However, it was successfully used in medical imaging applications [9]. Other applications of these techniques, such as determination of the material distribution and relaxation spectrum estimation, can be found in [4].

Problems with linear restoration of confocal images reside in the presence of ringing and other artifacts [5]. Better results can be obtained using non-linear algorithms, i.e. iterative methods based on maximum-likelihood or maximum a posteriori estimation. The most important iterative techniques are van Clittert's [1] and Richardson-Lucy [10] algorithms. The basic idea of van Clittert's method is to optimize some quality measure of the estimate by minimizing the difference between an estimated image and the measured image. The estimated image is created by means of a model based on the PSF of the microscope. This algorithm belongs to the deterministic deconvolution techniques. A similar technique, but a non-deterministic one, was proposed in Zimanyi [16].

The most popular method in the category of maximum likelihood (ML) estimation techniques is the Richardson-Lucy algorithm [10] which computes the unregularized ML estimator and therefore is sensitive to the noise realization. Numerous modifications and improvements of this algorithm exist [14], including those employing blind deconvolution.

PSF estimation is a non-trivial problem, since in CM it does not depend only on the microscope but it also depends on the measured object. Therefore, it is not sufficient to estimate just one PSF for the whole data volume, but specific PSFs for different substances or materials in the measures scene should be searched for [2].

3 THEORETICAL BACKGROUND

3.1 THE RICHARDSON-LUCY ALGORITHM

Maximum likelihood methods estimate the conditional probability $P(d|f)$, where d is the observed data and f is the object estimation. ML procedure finds the value of one or more parameters for a given statistics which maximizes the known likelihood distribution. [7].

In the following, we assume Poisson noise distribution of the observed data which can be expressed as $P(x,\lambda) = \frac{\lambda^x e^{-\lambda}}{x!}$, where λ is the expected number of events occurring during a fixed unit interval.

If the observed noisy data d is obtained by applying a Poisson distribution to f (i.e., image statistics is described as a Poisson process), we can set $x = d$, where $d = \{d_r, r \in \mathscr{S}\}$ and $\lambda = h \otimes f$, where h is PSF. The measured value d_r means photon count at any point r of definition range. Then, the likelihood probability could be expressed as [8, 10]

$$P(d|f) = \prod_{r\in\mathscr{S}} \left(\frac{((h\otimes f)_r)^{d_r} e^{-(h\otimes f)_r}}{d_r!} \right), \tag{1}$$

where $(h \otimes f)_r = \sum_{s\in\mathscr{S}} h_{r-s} f_s$ is a convolution for sites $\mathscr{S}$ ($\mathscr{S}$ is a definition range of the measured data d, in our case volume of size 1024x1024x27). It means that the photon count d_x inside a neighborhood $(x, \Delta x)$ of the point x is on average proportional to $f \otimes P(x|f)\Delta x$. The conversion of fluorescence intensity to a discrete number of detected photons is described as a translated Poisson process [14].

Solution of this problem is the well-known iterative form [8, 10]

$$f^{(n+1)} = f^{(n)} \left[h^T \otimes \left(\frac{d}{(h\otimes f)^{(n)}} \right) \right]; \text{ for } n \geq 1, \tag{2}$$

where h^T is transpose of h (if $h = h_{s-r}$ then $h^T = h_{r-s}$). The analogy of h^T in continuous form is an adjoint operator h^* and $h^*(x) = h(-x)$.

A problem of the R-L algorithm is that it does not have to converge when no regularization is used and the denominator has some zero values.

3.2 MAXIMUM A POSTERIORI METHODS

Let $f \subset \mathscr{L}$ is any set of values defined by $S_f \subset \mathscr{S}$, where $\mathscr{L}$ is a set of all possible vectors f (in our case it is a 3D volume and each element of this volume—voxel can have values from 0–256). We want to find such a vector f that the probability $P(f|d)$ is maximum. Hence, the solution is

$$f^* = \arg\max_{f\in\mathscr{L}} P(f|d). \tag{3}$$

This approach for estimating f^* by means of likelihood $P(f|d)$ is called a maximum a posteriori (MAP) estimation.

We can express the relationship between the measured data and the set of values f by the Bayes formula [7]:

$$P(f|d) = \frac{P(d|f)P(f)}{P(d)}, \tag{4}$$

where $P(f|d)$ is a conditional probability (a posterior probability), where d is fixed. Its maximization gives the solution, $P(d|f)$ is obtained by simulating the CM measurement procedure on the model f, $P(f)$ is a priori information about the object, obtained independently of the results of the measurement (in this paper it will be derived from the assumption of a MRF model) and, finally, $P(d)$ denotes probability of the single realization measured data (it is assumed to be a constant).

3.3 THE RICHARDSON-LUCY ALGORITHM WITH TIKHONOV-MILLER REGULARIZATION

A problem of the R-L algorithm resides in possible convergence to an unacceptable solution. An improvement can be achieved by adding prior model of the object, which represents a regularization term.

In 3D image restoration the Tikhonov-Miller (T-M) regularization [3, 14] is often used. The regularization term is used in the least square filter $||h \otimes \widehat{f} - d||^2$ between the acquired image d and a blurred estimation $\widehat{f}$ of the original object f. Finding the estimate $\widehat{f}$ is known to be an ill-posed problem [12] and the solution by minimization of this function is the well-known Tikhonov functional

$$\Phi(\widehat{f}) = ||h \otimes \widehat{f} - d||^2 + \lambda ||\mathbf{R}\widehat{f}||^2, \tag{5}$$

where $||\cdot||$ is the Euclidean norm, λ is a regularization parameter and $\mathbf{R}$ is a regularization matrix.

In order to get $||h \otimes \widehat{f} - d||$, we used simulation by Poisson probability density function (1). Then, we used logarithmic form of TM functional (5) and maximization of $P(f)$ is changed to minimization of L_{TM}. Hence,

$$L_{TM}(f) = \ln P(d|f) + \ln P(f) = \sum_{r \in \mathscr{S}} [-(h \otimes f)_r + d_r \ln(h \otimes f)_r)] + \lambda_{TM} \sum_{r \in \mathscr{S}} |\nabla f|^2, \tag{6}$$

where $\nabla f = f_i - f_{i-1}$. It depends on neighboring system.

From the ML principle, we can define new minimization functional as a differentiation of L by f

$$\frac{\partial L}{\partial f} = -\sum_{r \in \mathscr{S}} (h_{r-s}) + \sum_{r \in \mathscr{S}} d_r \frac{h_{r-s}}{(h \otimes f)_r} + 2\lambda_{TM} \sum_{s \in \mathscr{S}} |\nabla f| = 0; \text{ for each } s \in \mathscr{S}. \tag{7}$$

Inspired by (2), we get after minimization

$$f^{(n+1)} = \left[h^T \otimes \left(\frac{d}{(h \otimes f)^{(n)}} \right) \right] \frac{f^{(n)}}{1 + 2\lambda_{TM} \nabla f^{(n)}}; \text{ for } n \geq 1, \tag{8}$$

This formula is an iterative form of deconvolution algorithm called *Richardson-Lucy algorithm with Tikhonov-Miller regularization*. The algorithm has three input parameters: PSF h, measured data d and the coefficient λ_{TM}. The deconvolution process starts with some initial volume values (e.g. background values) and finishes after a finite number of iteration.

4 THE PROPOSED TECHNIQUE—IMPROVEMENT OF REGULARIZATION

Our goal is to estimate appropriate regularization parameters for different tissues and regions of the confocal data. From the MAP approach (3), we can see that a posteriori probability depends on both $P(f|d)$ and $P(f)$.

In the previous approach the Tikhonov functional was used as a regularization factor in $P(f)$. In confocal data deconvolution also other regularization forms as maximum entropy regularization [2] and Iterative Constrained Tikhonov-Miller deconvolution [6] are known. These regularizations do not take into account different types of structures present in the confocal data. Therefore we propose new regularization functions for different types of material structures (foggy objects, objects with a edges, etc), based on the MRF modeling.

We will now derive a general form of probability $P(f)$ by replacing the squared gradient in (6) by a general function $g(f)$:

$$L = \ln P(d|f) + \ln P(f) = \sum_{r \in \mathscr{S}} [-(h \otimes f)_r + d_r \ln(h \otimes f)_r)] + \lambda \sum_{r \in \mathscr{S}} g(f)_r. \tag{9}$$

Hence, the stationary points of L are the solutions of

$$\frac{\partial L}{\partial f} = -\sum_{r \in \mathscr{S}} (h_{r-s}) + \sum_{r \in \mathscr{S}} d_r \frac{h_{r-s}}{(h \otimes f)_r} + \lambda \sum_{r \in \mathscr{S}} \frac{\partial g(x)}{\partial f} = 0; \text{ for each } s \in \mathscr{S}. \tag{10}$$

Since PSF is normalized to 1, we can simplify (10) to

$$1+\lambda \sum_{r\in\mathscr{S}} \frac{\partial g(x)}{\partial f} = \sum_{r\in\mathscr{S}} d_r \frac{h_{r-s}}{(h\otimes f)_r}; \text{ for each } s\in\mathscr{S}. \tag{11}$$

Multiplying each of this equation by the related original data f yields:

$$f_s\left(1+\lambda \sum_{r\in\mathscr{S}} \frac{\partial g(x)}{\partial f}\right) = \sum_{r\in\mathscr{S}} d_r \frac{h_{r-s}f_s}{(h\otimes f)_r}; \text{ for each } s\in\mathscr{S}. \tag{12}$$

Using the EM algorithm, we get an iterative form

$$f_s^{(n+1)} = \left[\sum_{r\in\mathscr{S}} d_r \frac{h_{r-s}f_s^{(n)}}{(h\otimes f)_r^{(n)}}\right] \frac{1}{1+\lambda \sum_{r\in\mathscr{S}} \frac{\partial g(x)}{\partial f}}; \text{ for each } s\in\mathscr{S}, \tag{13}$$

and finally, utilizing a convolution form $\sum_{s\in\mathscr{S}} a_{r-s}b_s = (a\otimes b)_r$, we get

$$f^{(n+1)} = \left[h^T \otimes \left(\frac{d}{(h\otimes f)^{(n)}}\right)\right] \frac{f^{(n)}}{1+\lambda \sum_{r\in\mathscr{S}} \frac{\partial g(x)}{\partial f}}; \text{ for each } n\geq 1, \tag{14}$$

The function $g(x)$ in the MAP-MRF theory is called potential energy function [7].

4.1 POTENTIAL ENERGY FUNCTION

The potential function $g(x)$ is in general *piecewise continuous* and its set of values is *ordered*. It can significantly influence noise removal and edge preservation properties of the reconstruction process. For example, in the case of potential function $g(\eta) = |\eta|$, where $\eta = f_i - f_{i-1}$, noise has large influence on the deconvolution process. On the other hand, in the case of a potential function concave on the interval $(0,\infty)$, noise has smaller influence.

For the purpose of restoration, the function g is generally even $g(\eta) = g(-\eta)$ (due to the symmetry of f), non-negative on the interval $\langle 0,\infty)$ $g(\eta)\geq 0$ and non-decreasing $\eta_1 < \eta_2 \Rightarrow g(\eta_1)\leq g(\eta_2)$. Derivative of $g(\cdot)$ can be expressed as $g'(\eta) = 2\eta h(\eta)$. The function h_γ is called ***Adaptive iterated*** (AI) function and is usually parametrized by $\gamma > 0$. For further properties and requirements on h see [7].

As we have mentioned before, the range, where the function $g(x)$ is concave or convex is very important for the regularization process. By its specification we can design specific function for more or less noisy data, for foggy materials, or materials with edges. Table 1 presents the most common AI functions [7] and on the figure 1 are shown their geometric representation. Here, parameter γ defines interval B_γ, where function $g(\eta)$ is convex:

$$B_\gamma = \{\eta \mid g''_\gamma(\eta) > 0\} = (b_L, b_H). \tag{15}$$

Outside of this interval $g(\eta)$ is concave, which means that influence of differences of values $g(\eta_1)$ a $g(\eta_2)$ is suppressed and thus also is suppressed influence of noise on the restoration process.

5 CONCLUSIONS

We implemented both versions of the R-L algorithm, the original one with the T-M regularization (Eq. 8) and the new one with edge-preserving MRF regularization (Eq. 14) in the C++ language on a 2GHz AMD Sempron based PC equipped with 1GB of main memory.

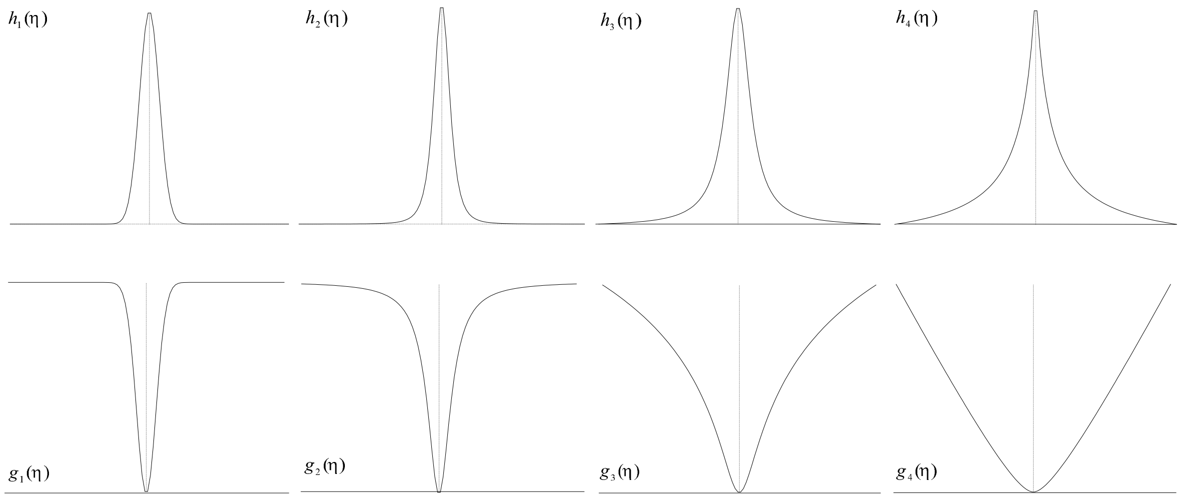

Figure 1: *AI function graphs (See Table 1)*

We used two data sets in testing. The first was a synthetic one of $256 \times 256 \times 256$ voxels with a voxelized hollow sphere [11] corrupted by Poisson noise (Fig 2). In this data set we focused on the ability to reconstruct homogeneous regions with sharp edges. As expected, we observed better results for less noisy data with potential functions g_4 and g_5 and for higher noise levels with functions g_3 and g_1. The lower row of Fig. 2 illustrates the results obtained with our new algorithm in comparison to the traditional reconstruction with the T-M regularization, where undesired blurring was observed. In both reconstructions 1000 iterations were used which took approximately 40 minutes.

The second data set was a stack of 10 slices of 512×2048 pixels (0.119×0.199 μm per pixel), depicting a cell, obtained by a confocal microscope. Here, no homogeneous areas were expected, since the chosen contrast agent accentuated membrane structures (Fig. 3). Therefore, the g_3 potential function was used for regularization.

In both cases the PSF was estimated from available parameters of the voxelization procedure (the sphere data set) and the scanning device (the cell data set). However, no exact measurements of PSF was involved. Therefore, our future work is to test the algorithms with estimates of real PSFs, to thoroughly evaluate influence of the potential functions in the case of different object types and

Table 1: AI functions

$h_{1\gamma}(\eta) = e^{-\frac{\eta^2}{\gamma}}$	$g_{1\gamma}(\eta) = -\gamma e^{-\frac{\eta^2}{\gamma}}$	$B_{1\gamma} = \left(-\sqrt{\frac{\gamma}{2}}, \sqrt{\frac{\gamma}{2}}\right)$
$h_{2\gamma}(\eta) = \frac{1}{(1+\frac{\eta^2}{\gamma})^2}$	$g_{2\gamma}(\eta) = -\frac{\gamma}{1+\frac{\eta^2}{\gamma}}$	$B_{2\gamma} = \left(-\sqrt{\frac{\gamma}{3}}, \sqrt{\frac{\gamma}{3}}\right)$
$h_{3\gamma}(\eta) = \frac{1}{1+\frac{\eta^2}{\gamma}}$	$g_{3\gamma}(\eta) = \gamma \ln\left(1 + \frac{\eta^2}{\gamma}\right)$	$B_{3\gamma} = \left(-\sqrt{\gamma}, \sqrt{\gamma}\right)$
$h_{4\gamma}(\eta) = \frac{1}{1+\frac{\lvert\eta\rvert}{\gamma}}$	$g_{4\gamma}(\eta) = \gamma\lvert\eta\rvert - \gamma^2 \ln\left(1 + \frac{\lvert\eta\rvert}{\gamma}\right)$	$B_{4\gamma} = \left(-\infty, \infty\right)$
$h_{5\gamma}(\eta) = \frac{1}{2\eta}$	$g_{5\gamma}(\eta) = \lvert\eta\rvert$	$B_{5\gamma} = \left(-\infty, \infty\right)$

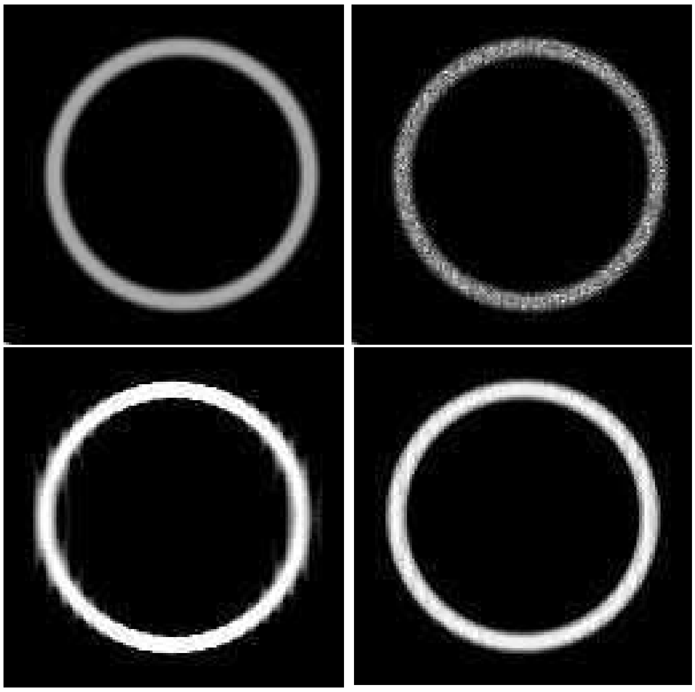

Figure 2: *One slice of the synthetic sphere data. Upper row: left—original, right—data corrupted by Poisson noise. Lower row: Deconvolution of the sphere data set by the R-L algorithm with T-M regularization (left) and our edge-preserving algorithm with using the function* g_4 *(right).*

finally, to optimize the algorithms to achieve better performance.

6 ACKNOWLEDGEMENT

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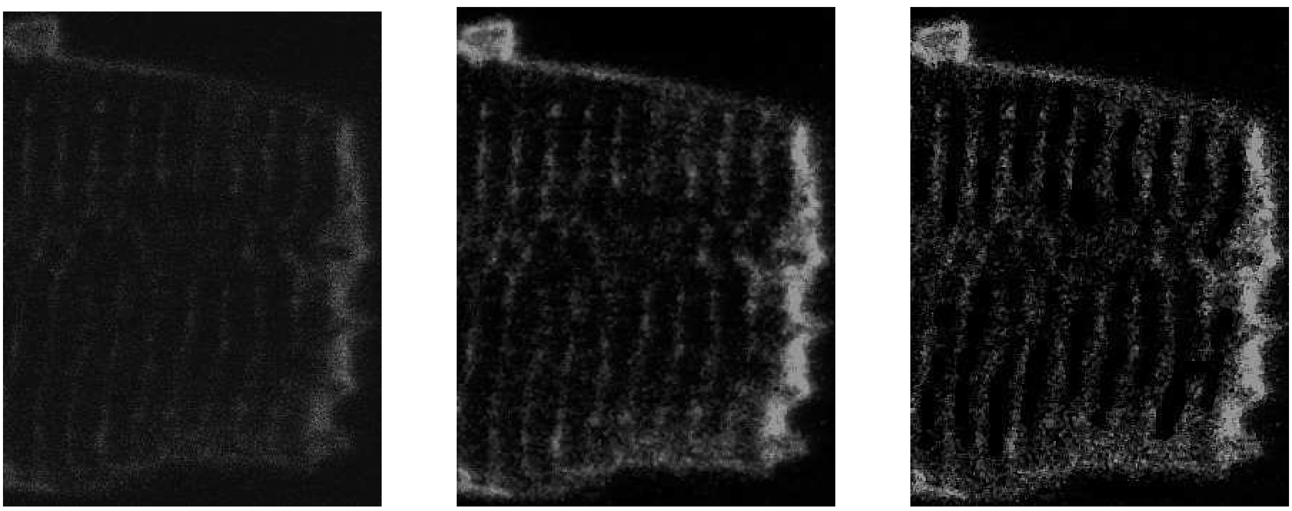

Figure 3: *The cell data. From left: original, T-M based deconvolution, our technique. With courtesy of Dr. Zahradnikova, Laboratory of Molecular Biophysics, Institute of Molecular Physiology and Genetics, SAS.*

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