

# **Histograms should not be used to segment hyperpolarized gas images of the lung**

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## Abstract

Magnetic resonance imaging using hyperpolarized gases has made possible the novel visualization of airspaces, such as the human lung, which has advanced research into the growth, development, and pathologies of the pulmonary system. In conjunction with the innovations associated with image acquisition, multiple image analysis strategies have been proposed and refined for the quantification of such lung imaging with much research effort devoted to semantic segmentation, or voxelwise classification, into clinically-oriented categories based on ventilation levels. Given the functional aspect of these images and the consequent sophistication of the segmentation task, many of these algorithmic approaches reduce the complex spatial image intensity information to intensity-only considerations, which can be contextualized in terms of the intensity histogram. Although facilitating computational processing, this simplifying transformation results in the loss of important spatial cues for identifying salient image features, such as ventilation defects (a well-studied correlate of lung pathophysiology), as spatial objects. In this work, we discuss the interrelatedness of the most common approaches for histogram-based segmentation of hyperpolarized gas lung imaging and evaluate the underlying assumptions associated with each approach demonstrating how these assumptions lead to suboptimal performance, particularly in terms of measurement precision. We then illustrate how a convolutional neural network can be trained to leverage multi-scale spatial information which circumvents the problematic issues associated with these approaches. Importantly, we provide the entire processing and evaluation framework, including the newly reported deep learning functionality, as open-source through the well-known Advanced Normalization Tools ecosystem (ANTsX).

# 1 Introduction

## 1.1 Early acquisition and development

Early hyperpolarized gas pulmonary imaging research reported findings in qualitative terms.

Descriptions:

- “<sup>3</sup>He MRI depicts anatomical structures reliably” (1)
- “hypointense areas” (2)
- “signal intensity inhomogeneities” (2)
- “wedge-shaped areas with less signal intensity” (2)
- “patchy or wedge-shaped defects” (3)
- “ventilation defects” (4)
- “defects were pleural-based, frequently wedge-shaped, and varied in size from tiny to segmental” (4)

## 1.2 Historical overview of quantification

Early attempts at quantification of ventilation images were limited to enumerating the number of ventilation defects or estimating the proportion of ventilated lung (4–6). This early work has evolved to current techniques which can be generally categorized in order of increasing algorithmic sophistication as follows:

- binary thresholding based on relative intensities (7, 8),
- linear intensity standardization based on global rescaling of the intensity histogram to a reference distribution based on healthy controls, i.e., “linear binning” (9, 10),
- nonlinear intensity standardization based on piecewise affine transformation of the intensity histogram using a customized hierarchical k-means algorithm (11, 12), and

- Gaussian mixture modeling (GMM) of the intensity histogram with Markov random field (MRF) spatial prior modeling (13)

where each of these algorithms has been purposely contextualized in terms of the intensity histogram for facilitating comparison.

An early semi-automated technique used to compare smokers and never-smokers relied on manually drawn regions to determine a threshold based on the mean signal and noise values (7). Related approaches, which use a simple rescaled threshold value to binarize the ventilation image into ventilated and non-ventilated regions (14), continue to find modern application (8). Similar to the histogram-only algorithms (i.e., linear binning and hierarchical k-means, discussed below), these approaches do not take into account the various MRI artefacts such as noise (15, 16) and the intensity inhomogeneity field (17) which prevent hard threshold values from distinguishing tissue types precisely consistent with that of human experts. In addition, to provide a more granular categorization of ventilation for greater compatibility with clinical qualitative assessment, many current techniques have increased the number of voxel classes (i.e., clusters) beyond the binary categories of “ventilated” and “non-ventilated.”

Linear binning is a simplified type of MR intensity standardization (18) in which a set of healthy controls, all intensity normalized to [0, 1], is used to calculate the cluster intensity boundary values, based on an aggregated estimate of the parameters of a single Gaussian fit. A subject image to be segmented is then rescaled to this reference histogram (i.e., a global affine 1-D transform). This mapping results in alignment of the cluster boundaries such that corresponding labels are assumed to have similar clinical interpretation. In addition to the previously mentioned limitations associated with hard threshold values, such a global transform does not account for MR intensity nonlinearities that have been well-studied (18–22) and are known to cause significant intensity variation even in the same region of the same subject. As stated in (21):

Intensities of MR images can vary, even in the same protocol and the same sample and using the same scanner. Indeed, they may depend on the acquisition conditions such as room temperature and hygrometry, calibration adjustment,

slice location, B0 intensity, and the receiver gain value. The consequences of intensity variation are greater when different scanners are used.

As we illustrate in subsequent sections, ignoring these nonlinearities are known to have significant consequences in the well-studied (and somewhat analogous) area of brain tissue segmentation in T1-weighted MRI (e.g., (23–25)) and we demonstrate its effects in hyperpolarized gas imaging quantification robustness in conjunction with noise considerations. In addition, the reference distribution required by linear binning assumes sufficient agreement as to what constitutes a “healthy control”, whether a Gaussian fit is appropriate, and, even assuming the latter, whether or not the parameter values can be combined in a linear fashion to constitute a single reference standard. Of more concrete concern, though, is that the requirement for a healthy cohort for determination of algorithmic parameters introduces a non-negligible source of measurement variance, as we will also demonstrate.

Previous attempts at histogram standardization (18, 20) in light of MR intensity nonlinearities have relied on 1-D piecewise affine mappings between corresponding structural features found within the histograms themselves (e.g., peaks and valleys). For example, structural MRI, such as T1-weighted neuroimaging, utilizes the well-known relative intensities of major tissues types (i.e., cerebrospinal fluid (CSF), gray matter (GM), and white matter(WM)), which characteristically correspond to visible histogram peaks, as landmarks to determine the nonlinear intensity mapping between histograms. However, in hyperpolarized gas imaging of the lung, no such characteristic structural features exist, generally speaking, between histograms. This is most likely due to the primarily functional utility (vs. anatomical) nature of these images. The approach used by some groups (11, 26) of employing some variant of the well-known k-means algorithm as a clustering strategy (27) to minimize the within-class variance of its intensities can be viewed as an alternative optimization strategy for determining a nonlinear mapping between histograms for a clinically-based MR intensity standardization. K-means does constitute an algorithmic approach with additional degrees of flexibility and sophistication over linear binning as it employs basic prior knowledge in the form of a generic clustering desideratum for optimizing a type of MR intensity standardization.<sup>1</sup>

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<sup>1</sup>The prior knowledge for histogram mapping is the general machine learning heuristic of clustering samples

Histogram-based optimization is used in conjunction with spatial considerations in the segmentation algorithm detailed in (13). Based on a well-established iterative approach originally used for NASA satellite image processing and subsequently appropriated for brain tissue segmentation in (28), a GMM is used to model the intensity clusters of the histogram with class modulation in the form of probabilistic voxelwise label considerations, i.e., MRF modeling, within image neighborhoods (29) using the expectation-maximization (EM) algorithm (30). Initialization for this particular application is in the form of k-means clustering. This has the advantage, in contrast to k-means and the other algorithms outlined, that it does not use hard intensity thresholds for distinguishing class labels which demonstrates robustness to certain imaging distortions, such as noise. However, as we will demonstrate, this algorithm is also flawed in that it implicitly assumes, incorrectly, that meaningful structure is found, and can be adequately characterized, within the associated image histogram in order to optimize a multi-class labeling. In particular, this algorithm is susceptible to MR nonlinear intensity artefacts.

Additionally, many of these segmentation algorithms use N4 bias correction (31), an extension of the nonuniform intensity normalization (N3) algorithm (17), to mitigate MR intensity inhomogeneity artefacts. Interestingly, N3/N4 also iteratively optimizes towards a final solution using information from both the histogram and image domains. Based on the intuition that the bias field acts as a smoothing convolution operation on the original image intensity histogram, N3/N4 optimizes a nonlinear (i.e., deformable) intensity mapping, based on histogram deconvolution. This nonlinear mapping is constrained such that its effects smoothly vary across the image. Additionally, due to the deconvolution operation, this nonlinear mapping sharpens the histogram peaks which presumably correspond to tissue types. While such assumptions are appropriate for the domain in which N3/N4 was developed (i.e., T1-weighted brain tissue segmentation) and while it is assumed that the enforcement of low-frequency modulation of the intensity mapping prevents new image features from being generated, it is not clear what effects N4 parameter choices have on the final segmentation solution, particularly for those algorithms that are limited to intensity-only considerations

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based on the minimizing within-class distance while simultaneously maximizing the between-class distance. In the case of k-means, this “distance” is the intensity variance.

and not robust to the aforementioned MR intensity nonlinearities.

### 1.3 Motivation for current study

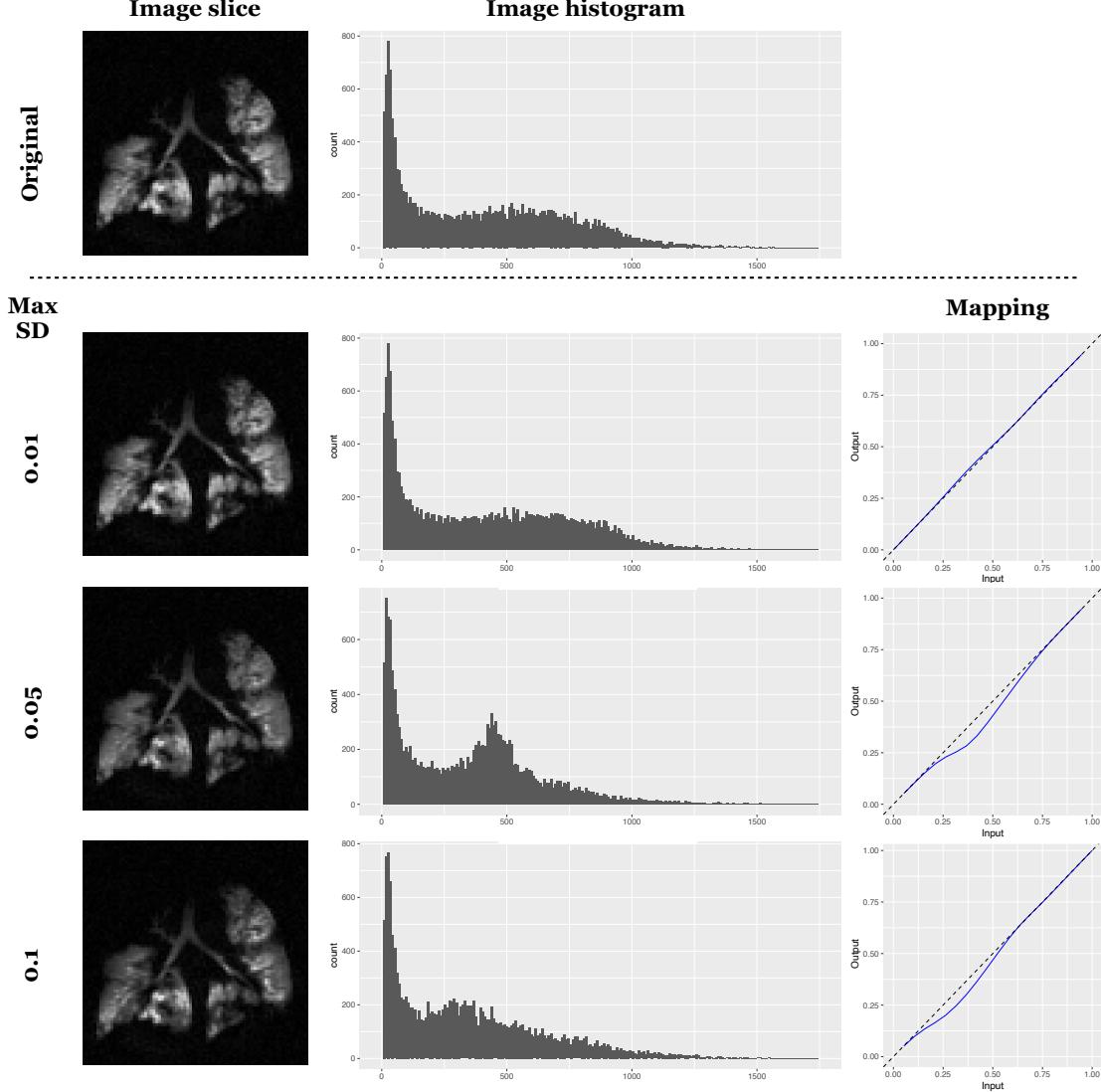


Figure 1: Illustration of the effect of MR nonlinear intensity warping on the histogram structure. We simulate these mappings by perturbing specified points along the bins of the histograms by a Gaussian random variable of 0 mean and specified max standard deviation (“Max SD”). By simulating these types of intensity changes, we can visualize the effects on the underlying intensity histograms and investigate the effects on salient outcome measures. Here we simulate intensity mappings which, although relatively small, can have a significant effect on the histogram structure.

Investigating the assumptions outlined above, particularly those associated with the nonlinear intensity mappings due to both the MR acquisition and inhomogeneity mitigation preprocess-

ing, we became concerned by the susceptibility of the histogram structure to such variations and the potential effects on current clinical measures of interest derived from these algorithms (e.g., ventilation defect percentage). Figure 1 provides a sample visualization representing some of the structural changes that we observed when simulating these nonlinear mappings. It is important to notice that even relatively small alterations in the image intensities can have significant effects on the histogram even though a visual, clinically-based assessment of the image can remain largely unchanged.

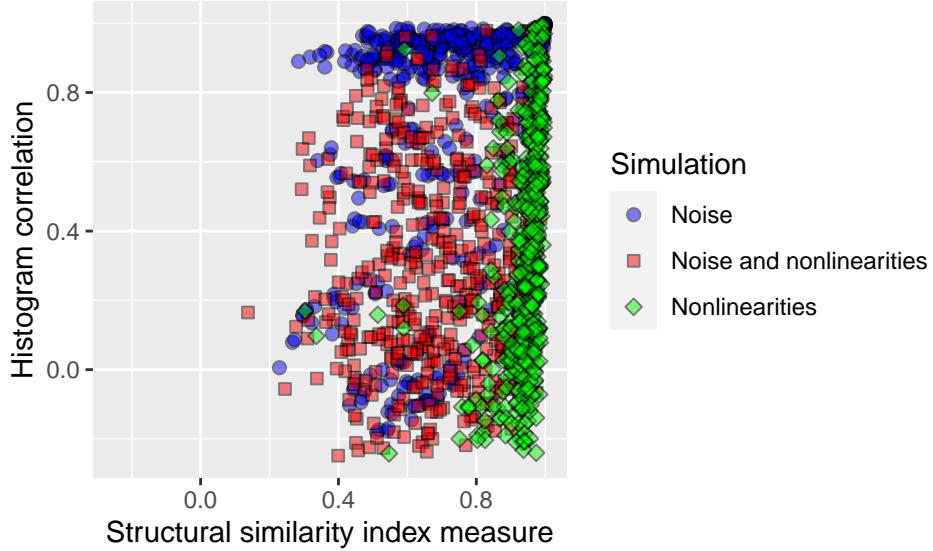


Figure 2: Image-based SSIM vs. histogram-based Pearson’s correlation differences under distortions induced by the common MR artefacts of noise and intensity nonlinearities. For the nonlinearity-only simulations, the images maintain their structural integrity as the SSIM values remain close to 1. This is in contrast to the corresponding range in histogram similarity which is much larger. Although not as great, the range in histogram differences with simulated noise is much greater than the range in SSIM. Both sets of observations are evidence of the lack of robustness to distortions in the histogram domain in comparison with the original image domain.

To briefly explore these effects further for the purposes of motivating additional experimentation, we provide a summary illustration from a set of image simulations in Figure 2 which are detailed later in this work and used for algorithmic comparison. Simulated MR artefacts were applied to each image which included both noise and nonlinear intensity mappings (and their combination) which made for a total simulated cohort of ~50 images ( $\times 10$  simulations per image  $\times 3$  types of artefact simulations). Prior to any algorithmic comparative analysis, we

quantified the difference of each simulated image with the corresponding original image using the structural similarity index measurement (SSIM) (32). SSIM is a highly-cited measure which quantifies structural differences between a reference and distorted (i.e., transformed) image based on known properties of the human visual system. SSIM has a range  $[-1, 1]$  where 0 indicates no structural similarity and 1 indicates perfect structural similarity. We also generated the histograms corresponding to these images. Although several histogram similarity measures exist, we chose Pearson's correlation primarily as it resides in the same  $[\min, \max]$  range as SSIM with analogous significance. In addition to the fact that the image-to-histogram transformation discards important spatial information, from Figure 2 it should be apparent that this transformation also results in greater variance in the resulting information under common MR imaging artefacts, according to these measures. Thus, prior to any algorithmic considerations, these observations point to the fact that optimizing in the domain of the histogram will be generally less robust than optimizing directly in the image domain.<sup>2</sup>

Ultimately, we are not claiming that these algorithms are erroneous, per se. Much of the relevant research has been limited to quantifying differences with respect to ventilation versus non-ventilation in various clinical categories and these algorithms have certainly demonstrated the capacity for advancing such research. However, the aforementioned issues influence quantitation in terms of core scientific measurement principles such as precision (e.g., reproducibility and repeatability (33)) and bias which become increasingly significant with multi-site (34) and large-scale studies. In addition, generally speaking, refinements in measuring capabilities correlate with scientific advancement so as acquisition and analysis methodologies improve, so should the level of sophistication and performance of the underlying measurement tools.

In assessing these segmentation algorithms for hyperpolarized gas imaging, it is important to

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<sup>2</sup>This point should be obvious even without the simulation experiments. Imagine, dear reader, the reality of the future clinical application of functional lung imaging beyond research activity. In fact, imagine yourself being a patient on the receiving end of an imaging battery which includes hyperpolarized gas imaging. Now imagine that, upon receiving the images for assessment, the radiologist declares “Yes, these are nice but I’d rather work with the corresponding histograms.” If this strikes you as absurd, then the point that we are trying to make should be clear.

note that human expertise leverages more than relative intensity values to identify salient, clinically relevant features in images—something more akin to the complex neural network structure versus the 1-D intensity histogram. The increased popularity of deep-layered neural networks (35), particularly convolutional neural networks (CNN), is due to their outstanding performance in certain computational tasks, including classification and semantic segmentation in medical imaging (36). Their potential for leveraging spatial information from images surpasses the perceptual capabilities of previous approaches and even rivals that of human raters (37). We introduced a deep learning approach in (38) and further expand on that work for comparison with existing approaches in this work. In the spirit of open science, we have made the entire evaluation framework, including our novel contributions, available within the Advanced Normalization Tools software ecosystem (ANTsX) (39).

## 2 Materials and methods

### 2.1 Hyperpolarized gas image cohort

A retrospective dataset was collected consisting of young healthy ( $n = 10$ ), older healthy ( $n = 7$ ), cystic fibrosis (CF) ( $n = 14$ ), interstitial lung disease (ILD) ( $n = 10$ ), and chronic obstructive pulmonary disease ( $n = 10$ ). Imaging with hyperpolarized  $^3\text{He}$  was performed under an Institutional Review Board (IRB)-approved protocol with written informed consent obtained from each subject. In addition, all imaging was performed under a Food and Drug Administration approved physician's Investigational New Drug application (IND 57866) for hyperpolarized  $^3\text{He}$ . MRI data were acquired on a 1.5 T whole-body MRI scanner (Siemens Sonata, Siemens Medical Solutions, Malvern, PA) with broadband capabilities and a flexible  $^3\text{He}$  chest radiofrequency coil (RF; IGC Medical Advances, Milwaukee, WI; or Clinical MR Solutions, Brookfield, WI). During a 10–20-second breath-hold following the inhalation of  $\approx 300$  mL of hyperpolarized  $^3\text{He}$  mixed with  $\approx 700$  mL of nitrogen, a set of 19–28 contiguous axial sections were collected. Parameters of the fast low angle shot sequence for  $^3\text{He}$  MRI were as follows: repetition time msec / echo time msec, 7/3; flip angle, 10°; matrix,  $80 \times 128$ ; field of view, 26  $80 \times 42$  cm; section thickness, 10 mm; and intersection gap, none. The data

were deidentified prior to analysis.

## 2.2 Algorithmic implementations

In support of the discussion in the Introduction, we performed various experiments to showcase the effects of both nonlinear intensity variation and noise artefacts on the resulting measurements using the algorithms described previously, specifically linear binning (9), hierarchical k-means (11), GMM-MRF (specifically, ANTs-based Atropos tailored for functional lung imaging) (13), and a trained CNN with roots in our earlier work (38), which we have dubbed “El Bicho”. A fair and accurate comparison between algorithms necessitates several considerations which have been outlined previously (40). In designing the evaluation study:

- All algorithms and evaluation scripts have been implemented using open-source tools by the first author. The linear binning and hierarchical k-means algorithms were recreated using existing R functionality. These have been made available as part of the GitHub repository corresponding to this work.<sup>3</sup> Similarly, N4, Atropos-based lung segmentation, and the trained CNN approach are all available through ANTsR/ANTsR-Net: `ANTsR::n4BiasFieldCorrection`, `ANTsR::functionalLungSegmentation`, and `ANTsRNet::elBicho`, respectively. Python versions are also available through ANTsPy/ANTsPyNet. The trained weights for the CNN are publicly available and are automatically downloaded when running the program.
- The imaging data used for the evaluation is available upon request and through a data sharing agreement. All other data, including additional evaluation plots are available, in the previously specified GitHub repository.
- An extremely important and characteristic hyperparameter is the number of ventilation clusters. In order to minimize differences in our set of evaluations and ensure a fair comparison, we optimized the segmentation based on the specified number of clusters. For the evaluations involving multiple algorithms, these were merged post-optimization to only three clusters: “ventilation defect,” “hypo-ventilation,” and “other ventilation”

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<sup>3</sup><https://github.com/ntustison/Histograms>

where the first two clusters for each output are the same as the original implementations and the remaining clusters are merged into a third category. It is important to note that none of the evaluations use these categorical definitions in a cross-algorithmic fashion. They are only used to assess within-algorithm consistency.

- One important issue was whether or not to use the N4 bias correction algorithm as a preprocessing step. We ultimately decided to include it for a couple reasons. It is explicitly used in multiple algorithms (e.g., (8, 9, 13)) despite the issues raised previously and elsewhere (10) due to the fact that it qualitatively improves image appearance.<sup>4</sup> Another practical consideration for N4 preprocessing was due to the parameters of the reference distribution required by the linear binning algorithm. Additional details are provided in the Results section.

## 2.3 Introduction of “El Bicho”

We extended the deep learning functionality first described in (38) to improve performance and provide a more clinically granular labeling (i.e., four clusters instead of two). In addition, further modifications incorporated additional data during training, added attention gating (41) to the U-net network (42) along with recommended hyperparameters (43), and a novel data augmentation strategy.

### 2.3.1 Network training

“El Bicho” is a 2-D U-net network was trained with several parameters recommended by recent exploratory work (43). The images are sufficiently small such that 3-D training is possible. However, given the large voxel anisotropy for much of our data (both coronal and axial), we found a 2-D approach to be sufficient. Nevertheless, a 2.5-D approach is an optional way to run the code for isotropic data where network prediction can occur in more than one slice direction and the results subsequently averaged. Four total network layers were employed with 32 filters at the base layer which was doubled at each subsequent layer.

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<sup>4</sup>This assessment is based on multiple conversations between the first author (as the developer of N4 and Atropos) and co-author Dr. Talissa Altes, one of the most experienced individuals in the field.

Multiple training runs were performed where initial runs employed categorical cross entropy as the loss function. Upon convergence, training continued with the multi-label Dice function (44)

$$Dice = 2 \frac{\sum_r |S_r \cap T_r|}{\sum_r |S_r| + |T_r|} \quad (1)$$

where  $S_r$  and  $T_r$  refer to the source and target regions, respectively.

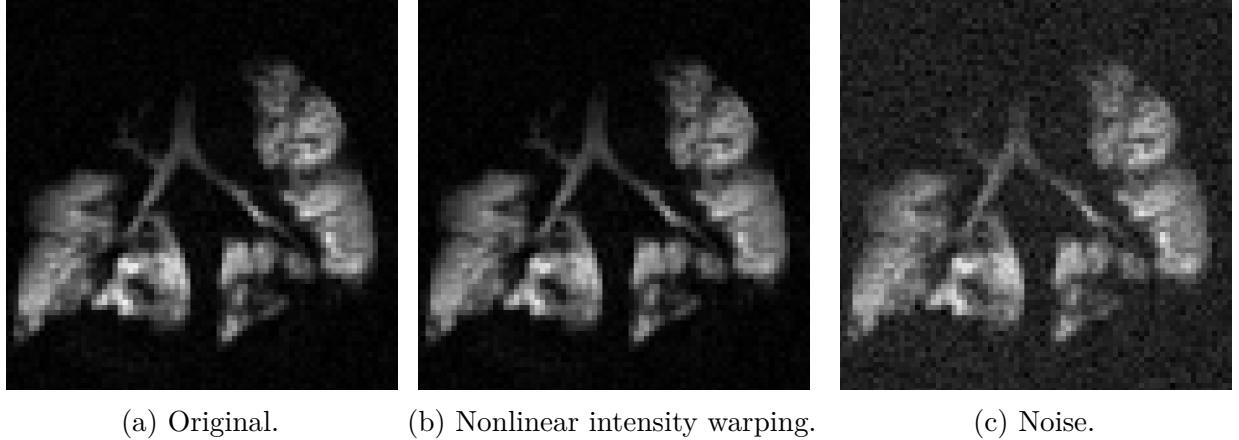


Figure 3: Custom data augmentation strategies for training to force a solution which focuses on the underlying ventilation-based lung structure. (b) Nonlinear intensity warping based on smoothly varying perturbations of the image histogram. (c) Additive Gaussian noise included for increasing the robustness of the segmentation network.

Training data (using an 80/20—training/testing split) was composed of the ventilation image, lung mask, and corresponding ventilation-based parcellation. The lung parcellation comprised four labels based on the Atropos ventilation-based segmentation (13). Six clusters were used to create the training data and combined to four for training the CNN. In using this GMM-MRF algorithm (which is the only one to use spatial information in the form of the MRF prior), we attempt to bootstrap a superior network-based segmentation approach by using the encoder-decoder structure of the U-net architecture as a dimensionality reduction technique. None of the evaluation data used in this work were used as training data. Responses from two subjects at the last layer of the network (with  $n = 32$  filters) are illustrated in Figure 4 which demonstrates the image-based approach to segmentation optimization.

A total of five random slices per image were selected in the acquisition direction (both axial and coronal) for inclusion within a given batch (batch size = 128 slices). Prior to slice extraction, both random noise and randomly-generated, nonlinear intensity warping was added to the 3-D image (see Figure 3) using ANTsR/ANTsRNet functions (`ANTsR::addNoiseToImage`, and `ANTsRNet::histogramWarpImageIntensities`) with analogs in ANTsPy/ANTsPyNet . 3-D images were intensity normalized to have 0 mean and unit standard deviation. The noise model was additive Gaussian with 0 mean and a randomly chosen standard deviation value between [0, 0.3]. Histogram-based intensity warping used the default parameters. These data augmentation parameters were chosen to provide realistic but potentially difficult cases for training. In terms of hardware, all training was done on a DGX (GPUs: 4X Tesla V100, system memory: 256 GB LRDIMM DDR4).

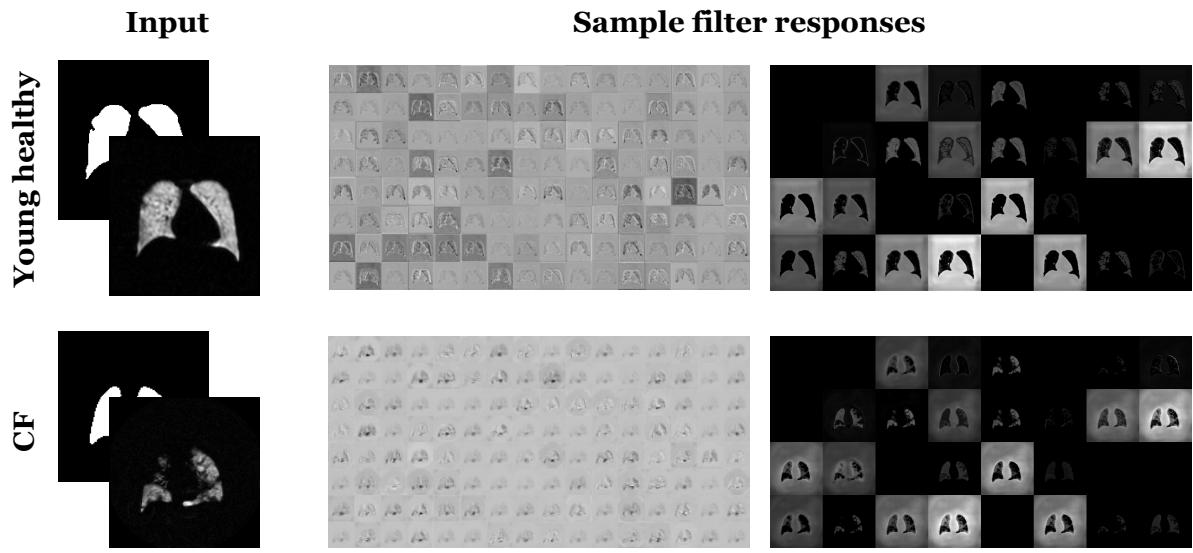


Figure 4: Optimized feature responses from both the encoding and decoding branches of the U-net network generated from a (top) young healthy subject and (bottom) CF patient. Note that these are optimized responses which take advantage of both the intensities and their spatial relationships.

### 2.3.2 Pipeline processing

An example R-based code snippet is provided in Listing 1 demonstrating how to process a single ventilation image using `ANTsRNet::elBicho`. If a simultaneous proton image has been acquired, `ANTsRNet::lungExtraction` can be used to generate the requisite lung mask input.

As mentioned previously, by default the prediction occurs slice-by-slice along the direction of anisotropy. Alternatively, prediction can be performed in all three canonical directions and averaged to produce the final solution.

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```

library( ANTsR )
library( ANTsRNet )

# Read in proton and ventilation images.
protonImage <- antsImageRead( "proton.nii.gz" )
ventilationImage <- antsImageRead( "ventilation.nii.gz" )

# Use deep learning lung extraction to get lung mask from proton image.
lungMask <- lungExtraction( protonImage, modality = "proton", verbose = TRUE )

# Run deep learning ventilation-based segmentation.
seg <- elBicho( ventilationImage, lungMask, verbose = TRUE )

# Write segmentation and probability images to disk.
antsImageWrite( seg$segmentationImage, "segmentation.nii.gz" )
antsImageWrite( seg$probabilityImages[[1]], "probability1.nii.gz" )
antsImageWrite( seg$probabilityImages[[2]], "probability2.nii.gz" )
antsImageWrite( seg$probabilityImages[[3]], "probability3.nii.gz" )
antsImageWrite( seg$probabilityImages[[4]], "probability4.nii.gz" )

```

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Listing 1: ANTsR/ANTsRNet command calls for processing a single ventilation image using El Bicho.

### 3 Results

We perform several comparative evaluations to probe the previously mentioned algorithmic issues which are broadly categorized in terms of measurement bias and precision, with most of the focus being on the latter. Given the lack of ground-truth in the form of segmentation images, addressing issues of measurement bias is difficult. In addition to the fact that the number of ventilation clusters is not consistent across algorithms, it is not clear that the ventilation categories across algorithms have identical clinical definition. This prevents application of various frameworks accommodating the lack of ground-truth for segmentation performance analysis (e.g., (45)) to these data.

As we mentioned in the Introduction, all the algorithms have demonstrated research (and potential clinical) utility based on findings using derived measures. This is supported by our first evaluation which is based on diagnostic prediction of given clinical categories assigned to the imaging cohort using derived random forest models (46). This approach also provides an

additional check on the validity of the algorithmic implementations. However, it is important to recognize that this evaluation is extremely limited as the underlying data are gross measures which do not provide accuracy estimates on the level of the algorithmic output (i.e., voxelwise segmentation).

Having established the general validity of the gross algorithmic output, we then switch to our primary focus which is the comparison of measurement precision between algorithms. We first analyze the unique requirement of a reference distribution for the linear binning algorithm. The latter is motivated qualitatively through the analogous application of T1-weighted brain MR segmentation. This component is strictly qualitative as the visual evidence and previous developmental history within that field should be sufficiently compelling in motivating subsequent quantitative exploration with hyperpolarized gas lung imaging. These qualitative results as a segue to quantifying the effects of the choice of reference cohort on the clustering parameters for the linear binning algorithm. We then incorporate the trained El Bicho model in exploring additional aspects of measurement variance based on simulating both MR noise and intensity nonlinearities.

So, in summary, we perform the following evaluations/experiments:<sup>5</sup>

- Global algorithmic bias (in the absence of ground truth)
  - Diagnostic prediction
- Voxelwise algorithmic precision
  - Three-tissue T1-weighted brain MRI segmentation (qualitative analog)
  - Input/output variance based on reference distribution (linear binning only)
  - Effects of simulated MR artefacts

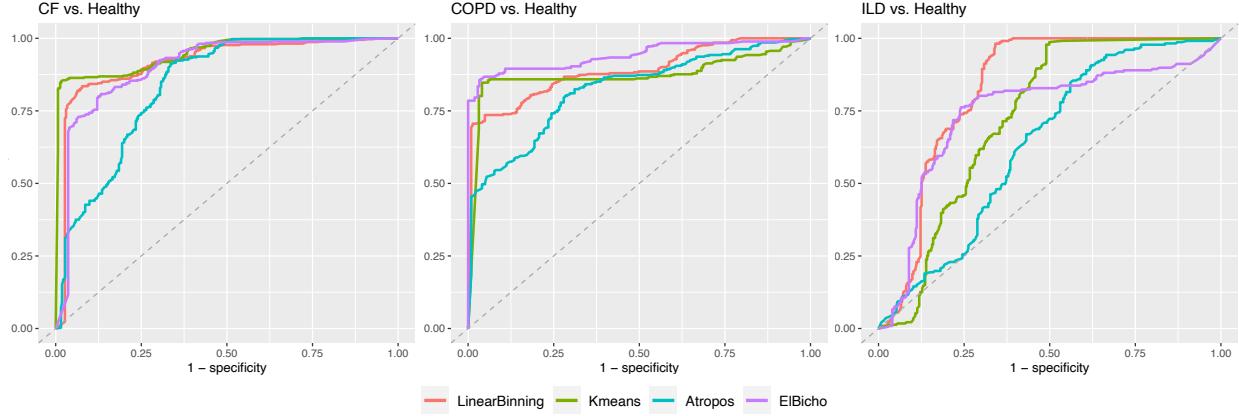


Figure 5: ROC curves resulting from the diagnostic prediction evaluation strategy involving randomly permuted training/testing data sets and predictive random forest models. Summary values are provided in Table 1.

### 3.1 Diagnostic prediction

Due to the absence of ground-truth but the availability<sup>5</sup>, we adopt the strategy from previous work (39, 47) where we used cross-validation to build and compare prediction models from data derived from the set of segmentation algorithms. Specifically, we use pathology diagnosis (i.e., “CF”, “COPD”, and “ILD”) as an established research-based correlate of ventilation levels from hyperpolarized gas imaging (e.g., (48–50)) and quantify the predictive capabilities of corresponding binary random forest classifiers (46) of the form:

$$\text{Pathology vs. Healthy} \sim \sum_{i=1}^3 \frac{\text{Volume}_i}{\text{Total volume}} \quad (2)$$

where  $\text{Volume}_i$  is the volume of the  $i^{th}$  cluster and  $\text{Total volume}$  is total lung volume. We used a training/testing split of 80/20. Due to the small number of subjects, we combined the young and old healthy data into a single category. 100 permutations were used where training/testing data were randomly assigned and the corresponding random forest model was constructed at each permutation.

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<sup>5</sup>It is important to note that, although these experiments provide supporting evidence, our principal contention stands prior to the results and are based on the self-evidentiary observations mentioned in the Introduction.

	<b>CF vs. Healthy</b>	<b>COPD vs. Healthy</b>	<b>ILD vs. Healthy</b>
<b>Linear binning</b>	0.92	0.89	0.83
<b>Hier. k-means</b>	0.95	0.87	0.73
<b>Atropos</b>	0.84	0.82	0.64
<b>El Bicho</b>	0.90	0.94	0.74

Table 1: AUC values describing the algorithmic performance for each set of binary classification simulations: CF vs. Healthy, COPD vs. Healthy, and ILD vs. Healthy. All four algorithms perform significantly better than a random classifier.

The resulting receiver operating characteristic (ROC) curves for each algorithm and each diagnostic scenario are provided in Figure 5. In addition, we provide the summary area under the ROC curve (AUC) values in Table 1. In the absence of ground truth, this type of evaluation does provide evidence that all these algorithms produce measurements which are clinically relevant although, it should be noted, that this is a very coarse assessment strategy given the global measures used (i.e., cluster volume percentage) and the general clinical categories employed.

### 3.2 T1-weighted brain segmentation analogy

Much of the quantitative image analysis strategies that have been used for hyperpolarized gas imaging draw on inspiration from fields with a much greater historical background of development, including T1-weighted brain MRI tissue segmentation. The depth of this development can be gauged simply by the number of technical reviews (e.g., (51–53)) and evaluation studies (e.g., (54, 55)) that date back decades. In addition to technical insight, this particular application provides a useful analogy for some of the algorithmic issues discussed and provides context for subsequent evaluations specific to hyperpolarized gas imaging.

In the style of linear binning, we randomly selected ten structurally healthy controls from the publicly available SRPB data set (56) comprising over 1600 participants from 12 sites. After intensity truncation at the 0.99 quantile, we normalize the intensity histogram to [0,1]. Eight of these histograms are provided in the upper left of Figure 6. As we mentioned previously, the histograms for these structural MRI are typically characterized by three peaks which correspond to the CSF, GM, and WM. However, even when normalized to [0, 1] (i.e., global

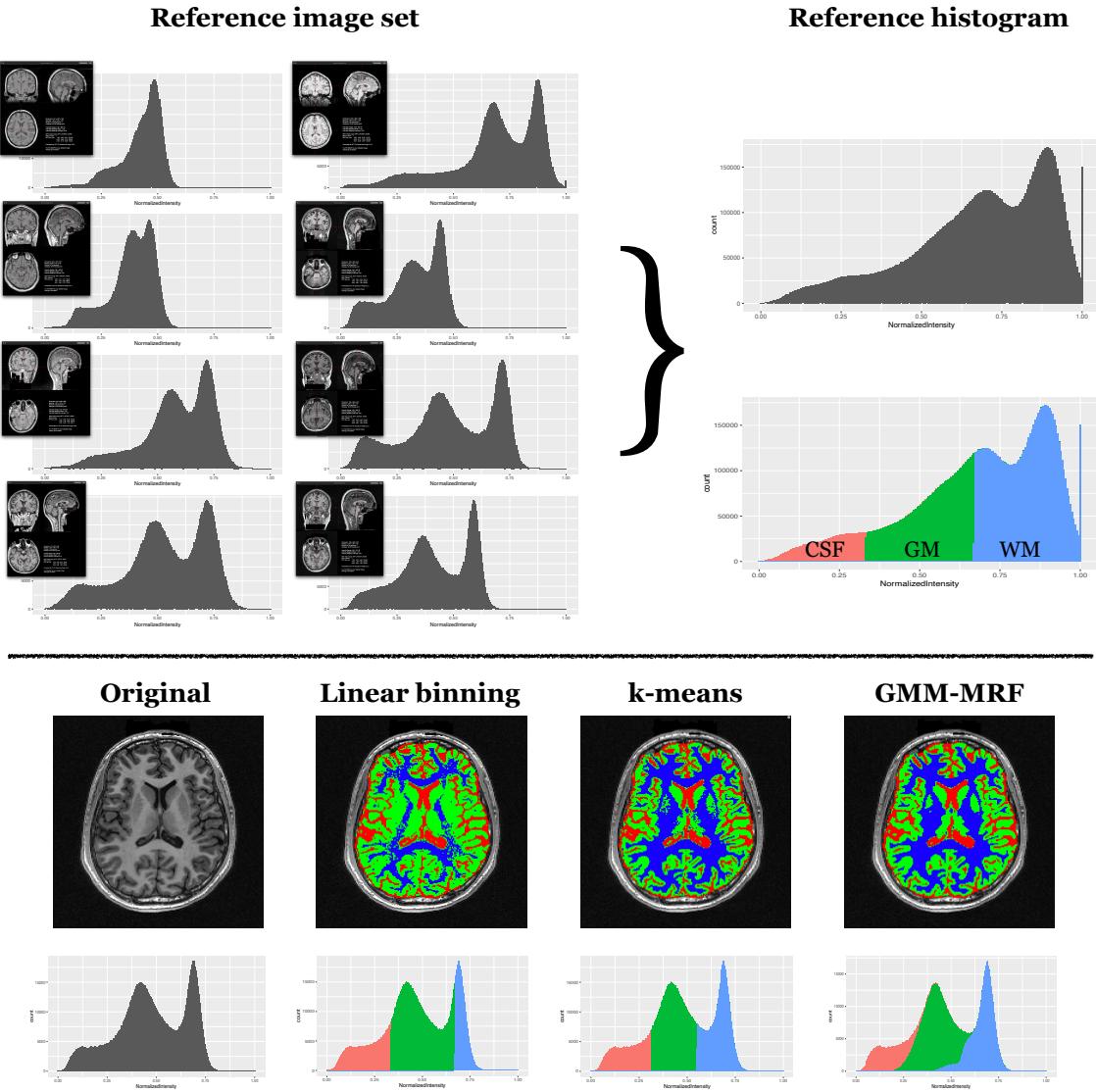


Figure 6: T1-weighted three-tissue brain segmentation analogy. Placing the three segmentation algorithms (i.e., linear binning, k-means, and GMM-MRF) in the context of brain tissue segmentation provides an alternative perspective for comparison. In the style of linear binning, we randomly select an image reference set using structurally normal individuals which is then used to create a reference histogram. (Bottom) For a subject to be processed, the resulting hard threshold values yield the linear binning segmentation solution as well as the initialization cluster values for both the k-means and GMM-MRF segmentations which are qualitatively different.

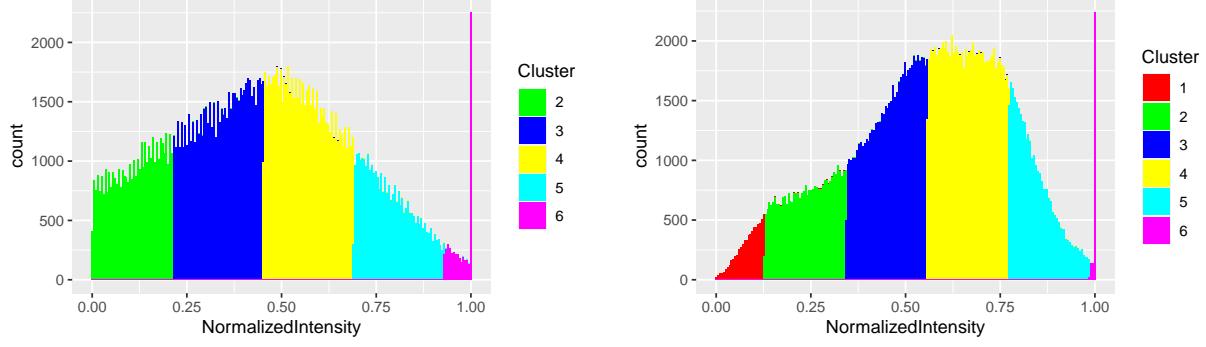
affine mapping), it is obvious that these histogram features do not line up and this is due to the intensity distortion caused by various MR acquisition artefacts mentioned previously. This is an argument from analogy against one of the principal assumptions of linear binning

where it is assumed that tissue types (“structural” in the case of T1-weighted brain MRI or “ventilated” in the case of hyperpolarized gas imaging) can be sufficiently aligned with a global rescaling of intensity values. If we pursue this analogy further and use the aggregated reference distribution to segment a different subject, we can see that, in this particular case, whereas the optimization criterion leveraged by k-means and GMM-MRF provide an adequate segmentation, the misalignment in cluster boundaries yield a significant overestimation of the gray matter content.

### 3.3 Effect of reference image set selection

One of the additional input requirements for linear binning over the other algorithms is the generation of a reference distribution. In addition to the output measurement variation caused by choice of the reference image cohort, this played a role in determining whether or not to use N4 preprocessing. As mentioned, a significant portion of N4 processing involves the deconvolution of the image histogram to sharpen the histogram peaks which decreases the standard deviation of the intensity distribution and can also result in an histogram shift. Using the original set of 10 young healthy data with no N4 preprocessing, we created a reference distribution according to (9), which resulted in an approximate distribution of  $\mathcal{N}(0.45, 0.24)$ . This produced 0 voxels being classified as belonging to Cluster 1 (i.e., ventilation defect) because two standard deviations from the mean is less than 0 and Cluster 1 resides in the region below -2 standard deviations. However using N4-preprocessed images produced something closer,  $\mathcal{N}(0.56, 0.22)$ , to the published values,  $\mathcal{N}(0.52, 0.18)$ , reported in (9), resulting in a non-empty set for that cluster. This is consistent, though, with linear binning which does use N4 bias correction for preprocessing.

The previous implications of the chosen image reference set also caused us to look at this choice as a potential source of both input and output variance in the measurements utilized and produced by linear binning. Regarding the former, we took all possible combinations of our young healthy control subject images and looked at the resulting mean and standard deviation values. As expected, there is quite a bit of variation for both mean and standard deviation values (see top portion of Figure 8 which are used to derive the cluster threshold



(a) Reference distribution (original images). (b) Reference distribution (N4 images).

Figure 7: Ten young healthy subjects were combined to create two reference distributions, one based on the (a) original images and the other using (b) N4 preprocessing. Based on the generated mean and standard deviation of the aggregated samples, we label the resulting clusters in the respective histograms. Due to the lower mean and higher standard deviation of the original image set, Cluster 1 is not within the range of [0, 1] for the resulting reference distribution which motivated the use of the N4-preprocessed image set.

values. This directly impacts output measurements such as ventilation defect percentage. For the reference sets comprising eight or nine images, we compute the corresponding linear binning segmentation and estimate the volumetric percentage for each cluster. Then, for each subject, we compute the min/max range for these values and plot those results cluster-wise on the bottom of Figure 8. This demonstrates that the additional requirement of a reference distribution is a source of potential measurement variation for the linear binning algorithm.

### 3.4 Effects of MR-based image distortions

## 4 Discussion

We recognize that alternative deep learning strategies (hyperparameter choice, training data selection, etc.) could provide comparable and even superior performance to what was presented. However, that is precisely our point—deep learning, generally, presents a much better alternative than histogram approaches as network training directly takes place in the image (i.e., spatial) domain and not in a transformed space where key information has been discarded.

As we mentioned previously, although susceptible to various levels of bias and lack of

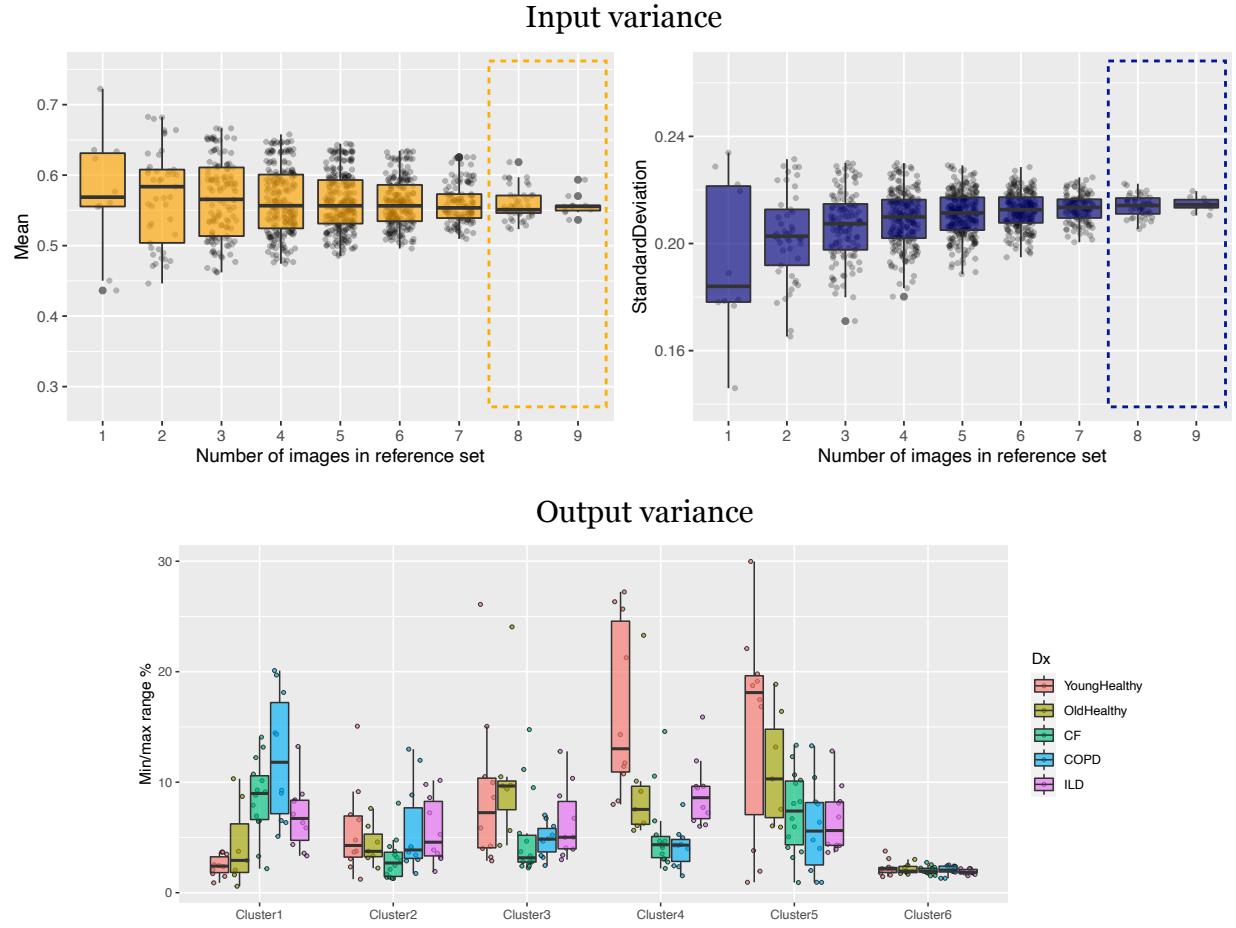


Figure 8: (Top) Variation of the mean (left) and standard deviation (right) over choice of reference set based on all different combinations of young healthy subjects per specified number of subjects. Although these parameters demonstrate convergence, there is still non-zero variation for any given set. (Bottom) This input variance is a source of output variance in the cluster volume plotted as the maximum range per subject as a percentage of total lung volume. We limit this exploration to reference sets with eight or nine images.

precision, these algorithms are decent for what they've been used for—global measurements, no more granular than spirometry, for doing research (while providing pretty visuals for publications.) However, if you want to do more sophisticated studies involving, for example, the spatial manifestation and/or growth of disease aided by advanced statistical techniques (such as similarity-driven multivariate linear reconstruction, then one should move beyond these shitty algorithms

not take advanta. For example, spirometry measures alone can be used to achieve highly

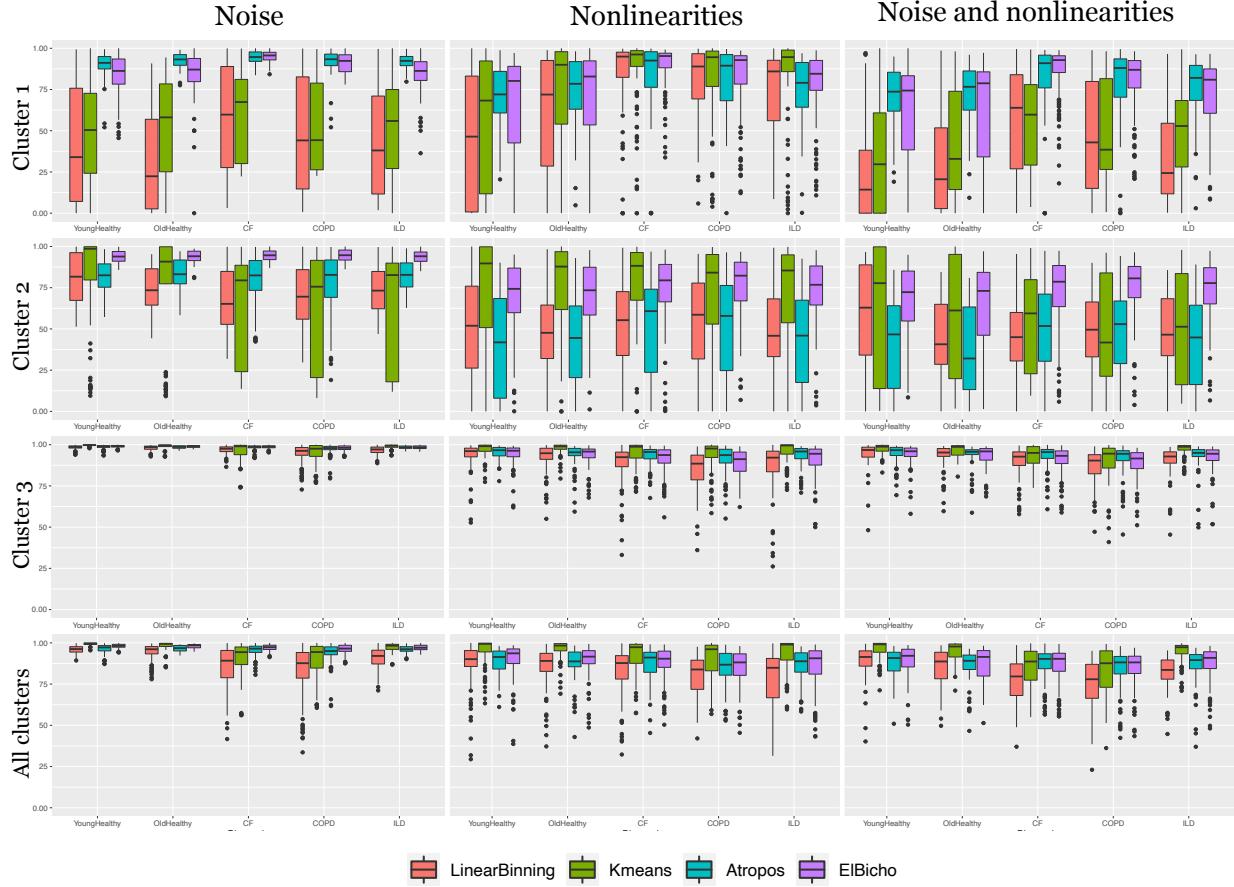


Figure 9: The deviation in resulting segmentation caused by distortions produced noise, histogram-based intensity nonlinearities, and their combination as measured by the Dice metric. Each segmentation is reduced to three labels for cross-comparison: “ventilation defect,” “hypo-ventilation,” and “other ventilation.”

accurate predictions using machine learning techniques (57).

In addition to the fundamental issues of precision and bias, we also point out that generally good modelling practice is to incorporate as much prior information as possible. Histogram-only algorithms throw out a significant portion of that prior information. This is a key consequence of the “No Free Lunch Theorem” (58)

There's other avenues to explore:

- the effects of super-resolution
- exploration of the trained weights for classification networks—what do they tell us

		Noise	Nonlinearities	Noise and nonlinearities
Cluster 1	<b>Linear binning</b>	0.46 ± 0.34	0.71 ± 0.31	0.4 ± 0.32
	<b>Hier. k-means</b>	0.54 ± 0.28	0.77 ± 0.32	0.47 ± 0.3
	<b>Atropos</b>	0.92 ± 0.06	0.78 ± 0.2	0.78 ± 0.19
	<b>El Bicho</b>	0.89 ± 0.11	0.78 ± 0.26	0.73 ± 0.26
Cluster 2	<b>Linear binning</b>	0.73 ± 0.17	0.51 ± 0.27	0.5 ± 0.24
	<b>Hier. k-means</b>	0.67 ± 0.34	0.75 ± 0.28	0.54 ± 0.33
	<b>Atropos</b>	0.81 ± 0.13	0.46 ± 0.3	0.44 ± 0.27
	<b>El Bicho</b>	0.94 ± 0.04	0.73 ± 0.21	0.71 ± 0.21
Cluster 3	<b>Linear binning</b>	0.97 ± 0.04	0.89 ± 0.12	0.91 ± 0.09
	<b>Hier. k-means</b>	0.97 ± 0.05	0.95 ± 0.07	0.94 ± 0.08
	<b>Atropos</b>	0.98 ± 0.02	0.94 ± 0.06	0.93 ± 0.07
	<b>El Bicho</b>	0.98 ± 0.01	0.92 ± 0.08	0.91 ± 0.08
All clusters	<b>Linear binning</b>	0.89 ± 0.11	0.83 ± 0.15	0.81 ± 0.13
	<b>Hier. k-means</b>	0.94 ± 0.09	0.93 ± 0.1	0.9 ± 0.11
	<b>Atropos</b>	0.96 ± 0.04	0.87 ± 0.1	0.86 ± 0.1
	<b>El Bicho</b>	0.97 ± 0.02	0.87 ± 0.11	0.87 ± 0.11

Table 2: Summary Dice values (mean ± standard deviation) for three sets of simulations over all four segmentation algorithms.

about disease?

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Instead of investing time in propping up shitty algorithms, we should be doing things like looking at tailored network architectures/features and data augmentation strategies.

So, in summary:

- In addition to completely discarding spatial information, linear binning is based on overly simplistic assumptions, especially given common MR artefacts. The additional requirement of a reference distribution, with its questionable assumption of Gaussianity, is also a potential source of output variance.
- Hierarchical k-means also ignores spatial information and, although it does use a principled optimization criterion, this criterion is not adequately tailored for hyperpolarized gas imaging and relatively more susceptible to various levels of noise than competing approaches.

- The GMM-MRF approach does employ spatial considerations in the form of Markov random fields but these are highly simplistic prior modeling of local voxel neighborhoods which do not capture the complexity of ventilation defects/heterogeneity appearance in the images. Although the simplistic assumptions provide some robustness to noise, the highly variable histogram structure in the presence of MR nonlinearities causes significant variance in the resulting GMM fitting.

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