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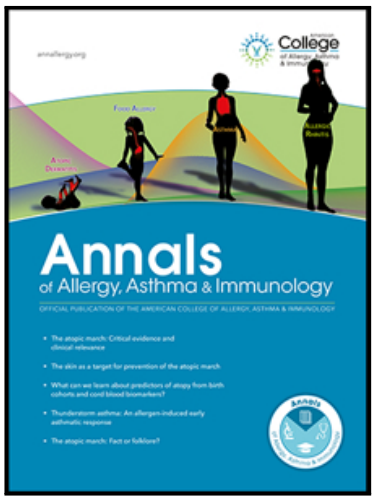

Variations in Ciliary Beat Frequency Based on Chronic Rhinosinusitis Endotype and Phenotype

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Title: Variations in Ciliary Beat Frequency Based on Chronic Rhinosinusitis Endotype and Phenotype

Running Title: Ciliary Beating and Chronic Rhinosinusitis Subtype

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Keywords: ciliary beat frequency, cilia, chronic rhinosinusitis with nasal polyps (CRSwNP), chronic rhinosinusitis without nasal polyps (CRSsNP), eosinophilic chronic rhinosinusitis (eCRS), non-eosinophilic chronic sinusitis (neCRS), spectrally encoded interferometric microscopy (SEIM)

Abbreviations:
CBF - ciliary beat frequency
CRS – chronic rhinosinusitis
CRSwNP - chronic rhinosinusitis with nasal polyps
CRSsNP - chronic rhinosinusitis without nasal polyps
eCRS - eosinophilic chronic rhinosinusitis
neCRS - non-eosinophilic chronic sinusitis
SEIM - spectrally encoded interferometric microscopy
SNOT-22 - Sinonasal Outcomes Test
PCM - phase contrast microscopy
OCT - optical coherence tomography
HBSS - Hank's balanced salt solution
FESS - functional endoscopic sinus surgery

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FPS - frames per second

FFT - fast Fourier transform

CF – Cystic fibrosis

PCD – Primary ciliary dyskinesia

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Abstract

Background: Chronic rhinosinusitis (CRS) is traditionally classified into CRS with or without nasal polyps and more recently into eosinophilic and non-eosinophilic endotypes. Limited research exists on the relationship between CRS subtype and mucociliary function. This study compares ciliary beat frequency (CBF) across CRS subtypes.

Objective: To investigate CBF across different CRS subtypes and validate spectrally encoded interferometric microscopy (SEIM) against phase contrast microscopy (PCM) for measuring CBF.

Methods: Sinonasal mucosa from endoscopic endonasal surgery cases were imaged ex vivo at physiologic temperature with PCM and SEIM. CBF measurements were compared between disease states (control vs chronic rhinosinusitis with nasal polyps (CRSwNP) vs chronic rhinosinusitis without nasal polyps (CRSsNP) and control vs eosinophilic chronic rhinosinusitis vs noneosinophilic chronic rhinosinusitis), as well as between PCM and SEIM.

Results: CRSwNP mucosa (5.77 ± 0.12 Hz) had significantly lower CBF compared to control (6.23 ± 0.11 Hz; p=0.001). Both eosinophilic rhinosinusitis (5.74 ± 0.16 Hz; p=0.005) and noneosinophilic CRS mucosa (6.00 ± 0.08 Hz; p=0.03) had significantly lower ciliary beat frequency compared to control (6.28 ± 0.11 Hz). There was no significant difference between PCM (7.65 ± 0.60 Hz) and SEIM (7.64 ± 0.51 Hz) as a means of evaluating CBF (p=0.36).

Conclusion: Among CRS subtypes, eosinophilic, noneosinophilic, and CRSwNP are associated with lower ciliary beat frequency when compared to healthy controls. SEIM may have value in measuring ciliary beat frequency.

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Introduction

The conducting respiratory system, from the nasal cavity to bronchioles, consists of ciliated pseudostratified columnar epithelial cells that are important for immunologic defense. Mucus in the upper airway traps dust, debris, and pathogens and is cleared by the constant rhythmic beating of cilia through a process known as mucociliary clearance.[1,2] Cilia are specialized structures composed of approximately 50 to 200 cilia per epithelial cell, each measuring 5 to 7 μm.[3] Coordinated ciliary beating is essential to mucus propulsion which can achieve transport velocities of 50 to 450 μm/s or 3 to 25 mm/min. Mucus is a viscoelastic substance with both liquid and solid properties. The viscosity of mucus decreases with increased ciliary beat frequency (CBF), enhancing its transport efficiency. Reduced ciliary beating subsequently may contribute to inspissated mucus characteristic of eosinophilic airway disease such as fungal allergic sinusitis.[4, 5] Ciliary function is impacted by the local climate, bacterial pathogens, allergens, and pharmaceutical agents, which in turn, affect mucous transport rate.[6-8] Ciliary disorders such as cystic fibrosis and primary ciliary dyskinesia have been the central focus of most ciliary function studies.[9-12] While ciliary dysfunction from sinonasal inflammatory disease such as chronic rhinosinusitis (CRS) has been studied, the relationship between CRS subtypes (endotype specifically) and ciliary dysfunction has been relatively overlooked.[13-23] This underscores the need to improve understanding and means of monitoring ciliary function.

Traditionally, CRS subtypes have been classified phenotypically into CRS with nasal polyps (CRSwNP) and without polyps (CRSsNP). Of these two phenotypes, CRSwNP has higher rates of recurrence and comorbid asthma, and is frequently refractory to treatment.[24-27] Recently, classification of CRS into endotypes has evolved based on inflammatory profile and cellular differentiation.[28-30] Differences exist in presentation, histopathology, and management of

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eosinophilic chronic rhinosinusitis (eCRS) versus non-eosinophilic rhinosinusitis (neCRS) in clinical practice.[31] Despite growing recognition of CRS endotypes as a key concept, there is a limited literature on the correlation between CRS subtypes and ciliary function.

The primary means of clinically evaluating sinonasal inflammatory disease traditionally include qualitative methods such as endoscopy and patient-reported outcome measures like the Sinonasal Outcomes Test (SNOT-22). These methods, however, have limitations in terms of standardization and direct quantification of mucociliary function. In addition to these, imaging modalities such CT and MRI, play a role in assessing the severity of CRS. These imaging modalities offer quantitative scoring of factors such as mucosal thickening, sinus opacification, the extent of multi-sinus involvement, and polyp severity. Despite their utility, these imaging techniques do not directly evaluate ciliary function, thus highlighting the need for improved methods to assess this aspect of sinonasal health.

Ciliary beat frequency (CBF) is a quantifiable measurement of ciliary health that is used to describe ciliary function.[8, 10] Currently, the most widely used method of evaluating ciliary beat frequency is high-speed video phase contrast microscopy (PCM).[32] However, this only images *ex vivo* tissue samples and can only measure CBF at the ciliated edges of tissue samples. CBF with PCM cannot capture ciliary behavior *in vivo* and does not allow for *en face* imaging, that is imaging across the entire two-dimensional (2D) tissue surface/plane. Recently, optical coherence tomography (OCT) has been used to image cilia and address some of the limitations of PCM.[9-11, 33] OCT imaging can be performed with an endoscopic probe that facilitates *in vivo* imaging. However, classic OCT technologies acquire only a cross-section of cilia across the mucosal surface and are unable to image tissue *en face*. To address this deficit, spectrally encoded

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interferometric microscopy (SEIM) was developed as a means of measuring ciliary beat frequency *en face* and generate 2D landscapes of CBF.[34-36]

This *ex vivo* study investigated CBF across different CRS phenotypes and endotypes to better understand the impact of sinonasal disease on ciliary function. Additionally, we sought to validate the use of SEIM against the gold standard PCM for measuring CBF. We hypothesized that patients with inflammatory subtypes of CRS would exhibit significantly lower CBF compared to healthy controls, indicating impaired mucociliary function. By exploring these objectives, our goal is to enhance the clinical evaluation of ciliary physiology and provide quantitative data for assessing sinonasal disease.

Methods

Ex vivo sample preparation

This study was approved by the University of California, Irvine (UCI) Institutional Review Board. Mucosal samples from primary or revision functional endoscopic sinus surgery (FESS), rhinoplasty, and skull base surgery cases were collected. Prior to surgery, all patients were treated with topical epinephrine via intranasal cottonoids for decongestion and hemostasis. Samples were surgical remnants from the inferior turbinate, middle turbinate, maxillary sinus, ethmoid sinus, sphenoid sinus, or uncinate process (Table 1) and removed with either straight or angled Blakesley forceps. The tissue was immediately placed in Hank's balanced salt solution (HBSS) without calcium and magnesium, maintained at physiologic temperature, and imaged within two hours of removal. Inferior turbinate (rhinoplasty with inferior turbinate reduction), uncinate process (FESS), and sphenoid sinus (endoscopic skull base surgery) tissue served as

controls.[37] Diagnosis of CRS was based on the American Academy of Otolaryngology-Head and Neck Surgery Clinical Practice Guideline definition.[38] Phenotype classification was based on the presence or absence of polyps during endoscopy while endotype classification was based on histopathologic evaluation of tissue (≥10 eosinophils per high powered field).[39] CRSwNP patients across this study cohort did not use biologics prior to surgery nor were on systemic medications at the time of tissue collection. CRSwNP patients may have been using topical nasal steroid spray or irrigations but were generally instructed to discontinue these one week prior to surgery. CRSsNP patients were not on medications prior to surgery and tissue collection.

Data acquisition

CBF acquisition with PCM was performed as previously described using an inverted phase contrast microscope (ID03, Zeiss, Oberkochen, Germany) with high-speed videography (iPhone 11 Pro, Apple, Cupertino, CA).[40] The tissue sample was placed in a glass-bottomed optical petri dish and covered with a layer of HBSS at physiologic temperature (37°C). The microscope stage was heated to maintain physiological temperature during imaging. High-speed video at 60 frames per second (FPS) recorded images of the ciliated edges of the mucosa using the 20x objective with 10 measurements for each tissue sample.

The SEIM system incorporated a swept-source laser (Thorlabs, Newton, NJ) and has a 100 kHz A-line rate and a 1.3 μm center wavelength as previously described by our group.[34-36, 41] The system can resolve *en face* displacement up to 100 frames per second with 1000 A-lines per image with a lateral resolution and axial displacement sensitivity of 1.2 μm and 0.3 nm, respectively. This system was used to visualize the mucosal surface and captured at least 10

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patches of ciliated cells from which CBF data was collected. To validate the CBF values from SEIM, the same region of cilia on tissue samples was imaged with both SEIM and PCM.

Computation Analysis

Code written in the MATLAB programming environment (Mathworks, Natick, MA) processed the high-speed PCM video. A 5x5 pixel area of cilia was selected in the software, creating a region of interest (ROI). A fast Fourier transform (FFT) was performed on this ROI to extract frequency information from the signal. From this, a power spectrum and time domain graph were created to identify the ciliary beat frequency.

To acquire the CBF from SEIM, data was processed by first performing a Hilbert transform on the raw signal to yield the analytical signal. The phase component was extracted from the analytical signal and a Doppler phase-resolved algorithm was used to acquire the displacement of the sample at a given location. This resulted in a raw spatial Doppler phase map that was analyzed over time. Using this phase map, the cilia was graphed out over time and an FFT was used to obtain the frequency spectrum which provided the CBF at that location (Figure 1).

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Statistical Analysis

All statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS) (IBM, Armonk, NY). Statistical significance was set at $p<0.05$ for all tests. A generalized linear mixed model was used to compare the CBF of patients based on phenotype (control vs.

CRSwNP vs. CRSsNP) and based on endotype (control vs. eCRS vs. neCRS). To validate the SEIM device, the mean CBF values of the control using both PCM and SEIM methods were compared using a Wilcoxon signed rank test.

Results

Tissue samples included in this study were mostly collected from female patients (39 mucosal samples, 54.6%). Median age at time of surgery was 57 years (IQR 32.5-64.0) (Table 1). Mucosal samples were obtained from functional endoscopic sinus surgery (26 cases, 36.6%), endoscopic skull base surgery (20 cases, 28.2%), rhinoplasty with turbinate reduction (20 cases, 28.2%), and resection of sinonasal tumors (5 cases, 7.0%). Across the study cohort, 45 (63.4%) of the mucosal samples were obtained from healthy controls, 11 (15.5%) from patients with CRSwNP, and 15 (21.1%) from patients with CRSsNP. When describing CRS endotype, 19 (26.7%) mucosal samples came from patients with neCRS, and 7 (9.9%) from patients with eCRS patients. There were no smokers in this study cohort. Descriptive statistics of ciliary beat frequency across phenotype, endotype, and location are included in Table 2.

Across CRS phenotypes, patients with CRSwNP (5.77 ± 0.12 Hz) (mean ± standard error) had a lower mean CBF compared to the healthy control (6.28 ± 0.11 Hz; p=0.001). However, the mean CBF of patients with CRSsNP (6.05 ± 0.09 Hz) was not significantly different from that of the healthy control patients (p=0.1) (Figure 2A). Across CRS endotypes, both patients with eCRS (5.74 ± 0.16 Hz; p=0.005) and neCRS (6.00 ± 0.08 Hz; p=0.03) had significantly lower CBF when compared to controls (6.28 ± 0.11 Hz) (Figure 2B). The mean CBF of the control as determined through PCM was 7.65 ± 0.60 Hz while the mean CBF of the

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control as determined through SEIM was 7.64 ± 0.51 Hz (Figure 3). There were no significant differences between these two imaging modalities (p=0.36).

Discussion

This study provides critical insights into the relationship between CRS subtypes and ciliary function, as indicated by CBF. Our findings indicate that CBF in patients with CRSwNP and both eCRS and neCRS endotypes are significantly lower compared to controls, and suggest inflammatory processes associated with CRS may impair ciliary function. This study also validated SEIM against the gold standard PCM, showing no significant difference, thus supporting SEIM as a reliable tool for assessing CBF.

While several studies have highlighted the relationship between CRS and both mucociliary clearance and CBF, there is limited literature on the correlation between CRS subtype and sinonasal CBF.[21, 42-46] A study by Li et al. found a significantly lower CBF across patients with nasal polyps when compared to healthy patients, and hypothesized that overly dense, lengthened, and untidy organization of cilia due to chronic inflammation and/or infection found in patients with nasal polyps were the cause.[15] These findings align with our study, which showed significantly lower CBF in sinonasal mucosa from CRSwNP patients compared to controls. Given the findings by Li et al., this significant decrease is likely secondary to the greater degree of abnormal cilia architecture in the CRSwNP phenotype; Li et al. postulate that functional changes to the epithelium may result in abnormal regulation of cilia assembly. Other studies looking at change in CBF after surgical and antibiotic treatment of CRS showed increase CBF after treatment,[22, 47] hence polypoid mucosal change may lead to inflammatory processes

that promote undesirable cilia architecture. These findings underscore the strong and consistent association between CBF and CRS phenotype-related pathophysiology observed in our study.

In the analysis across CRS endotypes, CBF was lower in both eCRS and neCRS subtypes compared to healthy controls. Current literature suggests that eCRS disease is associated with Type 2 inflammation and eosinophilic infiltration while neCRS is more strongly associated with neutrophilic infiltration.[31] Although the histopathology of these two CRS endotypes differ, mucosal inflammation is a common end pathway and may contribute to abnormal cilia function and decreased CBF as observed in this study. This impaired mucociliary function may contribute to the disease severity and symptom burden in CRS patients.

Outside of CRS, there is a modest body of literature describing CBF in diseases such as cystic fibrosis (CF) and primary ciliary dyskinesia (PCD) which may be associated with decreased CBF.[9-11, 48-50] PCD has been shown to result in decreased CBF due to ultrastructural ciliary defects. A study by Chilvers et al. demonstrated that, in patients with PCD, cilia with outer dynein arm or with outer and inner dynein arm defects had mean CBF of 2.3 Hz and 0.8 Hz, respectively.[48] Chilvers et al. found that these ultrastructural defects responsible for PCD may result in predictable decreases in beat frequency which may aid in diagnostic evaluation. Regarding CF, there is uncertainty in the literature whether CF consistently is associated with decreased CBF. Some studies have found no significant changes in CBF in CF while others have observed decreases in CBF and have even suggested that CBF may serve as a means of quantifying the therapeutic effects of pharmacologic agents on epithelial cell function.[49-53] The utilization of CBF across PCD and CF highlight the potential for CBF measurement as a diagnostic tool and measure of disease burden across ciliary diseases.

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Establishing the range of normal CBF values, mapping them with respect to anatomic location, and identifying patients whose values are out of range may have profound implications for precision medicine. For instance, knowing the typical CBF range in healthy individuals allows for the identification of variance that may by indicative of specific pathological conditions. Lower CBF in asymptomatic individuals may serve as an early indicator of an underlying pathology, even before clinical symptoms of CRS manifest. Understanding the mechanisms of these disease processes and how they impact mucociliary function and disease severity has the potential to help better address disease management and diagnosis while providing clinically relevant references for CBF. To our knowledge, this is the first study looking at CBF across specific CRS endotypes (i.e., eCRS and neCRS). Decreased CBF across CRSwNP, eCRS and neCRS compared to controls suggests that measurement of CBF may have utility among CRS subtypes with nasal polyps, possibly having implications in optimizing diagnostic and treatment strategies. Further investigations should continue to study CBF across phenotypes and endotypes of CRS while correlating CBF with other measures such as SNOT-22, endoscopy, and radiographic measures.

Admittedly, there is great deal of variation in CBF values across the literature which may in part be attributed to factors such as the methodology utilized. Due to technologic limitations, measurement of CBF is largely done *ex vivo*, introducing variability in how the tissue is sampled and measured. While some studies have utilized nasal swab brushings to collect ciliated cells for analysis, other studies utilize mucosal samples collected during surgery. A study by Thomas et al. looked at the association between sinonasal CBF and mucosal damage during biopsy.[54] They found a significant decrease in CBF as the level of mucosal damage increased. This likely plays a strong role in the variance in CBF found across studies. For example, Ho et al. utilized a

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cytology brush swab to sample mucosa from healthy patients which had a median ± SD CBF of 13.0 ± 1.7 Hz.[55] A study by Jorissen et al., sampled sinonasal tissue surgically and found a CBF of 7.0 ± 2.6 Hz, lower than that found by Ho et al.[56] These tissue collection methods introduce variability due to the *ex vivo* measurement of these mucosal samples. However, *in vivo* measurement of healthy sinonasal CBF, although not as common in the literature, reported a mean CBF of 14.4 Hz.[9] The CBF values in our study were most similar to the reported values from Jorissen et al., which may be due to the use of surgical removal of tissue rather than nasal swab brush biopsy. While not entirely representative of the body of literature describing sinonasal CBF values, these studies illustrate the variability in CBF measurements across differing methods for collecting mucosal samples. Further contributors to variability include temperature control of samples, anesthetic utilized for tissue biopsy, and potential differences in CBF measurement software for PCM. This highlights the need for a CBF measurement method that reduces these effects and standardizes technique.

While PCM has been the gold standard of measuring CBF, OCT has been utilized in recent years to study and image cilia.[9, 11, 33, 34, 36] Oldenberg et al. developed a reliable method for measuring CBF by analyzing the median frequency of a power spectrum obtained from speckle fluctuations in OCT images.[57] Since then, other methods have expanded on this approach, providing quantifiable, and standardized processes. Development of an SEIM system utilizing technology similar to OCT has been described in previous studies.[34-36] Our study demonstrates that SEIM is a valid method of measuring CBF of sinonasal cilia as SEIM and PCM values matched. The use of SEIM has advantages over PCM. *En face* imaging of cilia allows for the imaging of the metachronal wave propagation which is a characteristic of cilia that is poorly studied and also allows for a better capturing of ciliary heterogeneity across the mucosal

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surface.[35] SEIM technology can also be configured in a handheld endoscope that gives it the potential to image cilia *in vivo* in clinical settings. Nasal endoscopy and patient-reported outcome measures lack standardized and directly quantifiable evaluation of cilia. The use of SEIM has the potential to address the need for better assessments of mucosal health and the evaluation of cilia. Following this validation of SEIM technology, future studies should study the association between *in vivo* CBF and factors that have been shown to affect CBF *ex vivo*.

A limitation of this study was the lack of a standardized protocol for pretreatment of the nose during surgery. Topical and injected lidocaine and epinephrine were used at the beginning of the operative cases without a standardized procedure for this study but rather were administered in doses based on clinical preference of the surgeon. Although the time between application of these agents, biopsy, and imaging of cilia was typically greater than 2 hours, the potential impact of these agents on CBF was not entirely standardized and remains a limitation of the study. Our study provides insight into the role of ciliary function in CRS but is limited by the absence of controlling for other common inflammatory conditions such as allergic and vasomotor rhinitis. Specifically studying groups with these conditions could help elucidate the effects of baseline upper airway inflammation on cilia and could offer a better understanding of the pathophysiologic changes specific to CRS. Future studies should investigate the effects of allergic and vasomotor rhinitis on CBF with our methodology. Additionally, future studies can investigate the association of decreased CBF with clinical disease burden as reported by clinical outcome measures and treatment efficacy. Another limitation includes the modest sample size of this study which should be addressed in future studies to increase the statistical power to further validate these findings. Additionally, histopathologic evaluation of mucosal samples was not conducted by a single pathologist which may have introduced variability in the endotype

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diagnosis of tissue samples. Future studies should utilize further histopathologic evaluation of tissue samples, including but not limited to the measurement of CRS biomarker and cytokine levels. Additionally, this study was limited to a cohort of non-smoking patients. While this eliminated a potential confounding variable, it should be acknowledged that cigarette smoke does inflame mucosa in a manner that may also affect ciliary beat frequency.[58] Future studies should be conducted to further investigate the association between these characteristics and CBF.

Conclusion

In summary, this study addresses the paucity of literature on CRS subtypes and mucociliary function as measured by CBF and validates the use of SEIM as a reliable method for CBF assessment. These findings suggest that patients with CRSwNP and both eosinophilic and non-eosinophilic CRS endotypes exhibit significantly lower CBF compared to healthy controls. This underscores the potential impact of inflammatory processes on ciliary function as reflected by CBF. The validation of SEIM against the gold standard PCM highlights its potential for *in vivo* application, offering a novel and quantifiable approach to evaluating ciliary health and enhancing the clinical assessment of sinonasal disease. Future research should continue to explore the utility of SEIM in CRS, aiming to refine diagnostic and therapeutic strategies across CRS phenotypes and endotypes.

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Figure

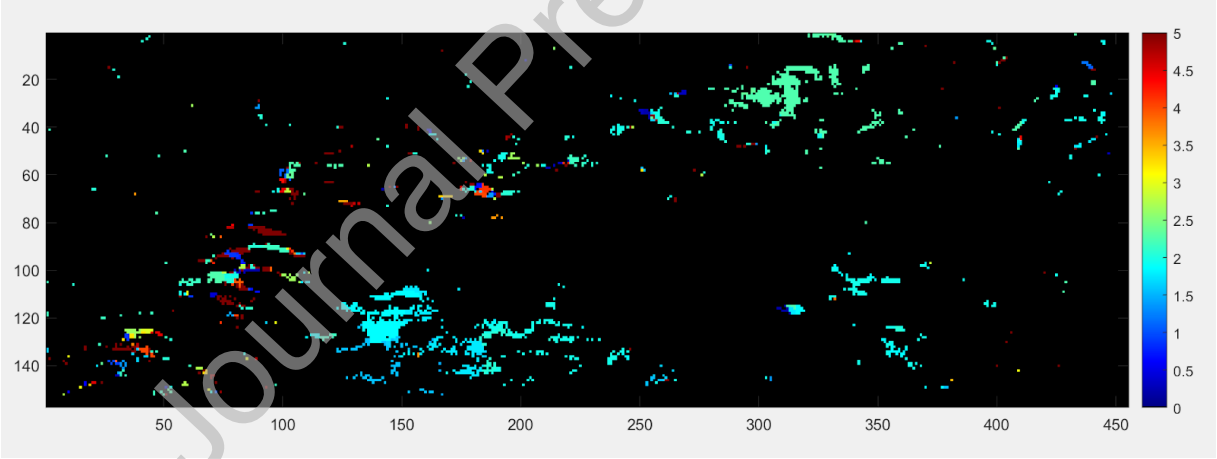

Figure 1

Figure 1: Representative map of ciliary beat frequencies acquired across a tissue surface with spectrally encoded interferometric microscopy (SEIM). The color legend on the right-hand side corresponds with beat frequencies. The x and y-axes represent arbitrary coordinate values.

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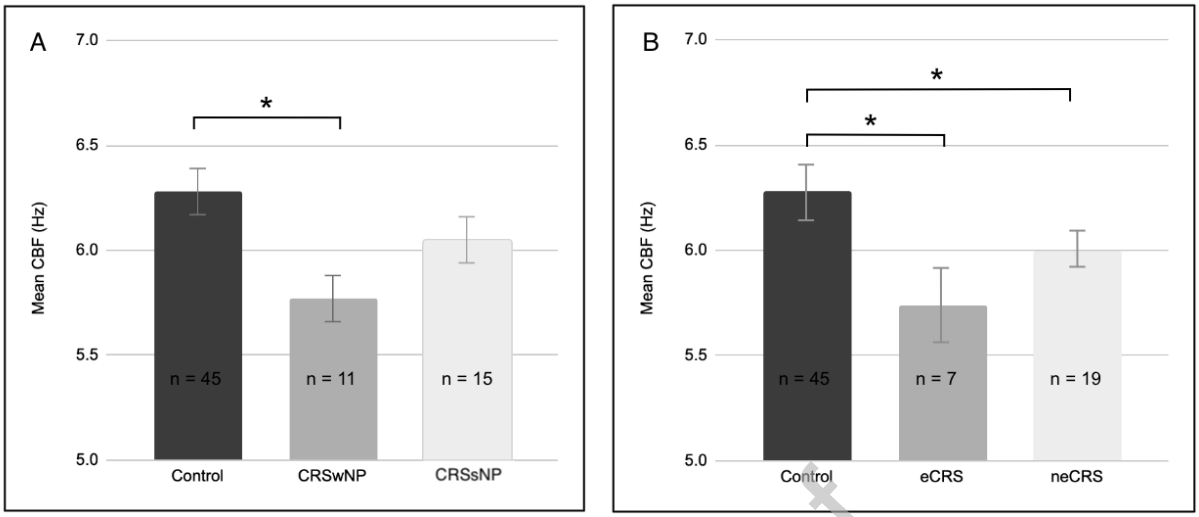

Figure 2

Figure 2: (A) Mean CBF and standard error for healthy control (6.28 ± 0.11 Hz), chronic rhinosinusitis with nasal polyps (CRSwNP) (5.77 ± 0.12 Hz), and chronic rhinosinusitis without nasal polyps (CRSsNP) (6.05 ± 0.09 Hz). (B) Mean CBF and standard error for healthy control (6.28 ± 0.11 Hz), eosinophilic CRS (eCRS) (5.74 ± 0.16 Hz), and noneosinophilic CRS (neCRS) (6.00 ± 0.08 Hz) (* = significant difference).

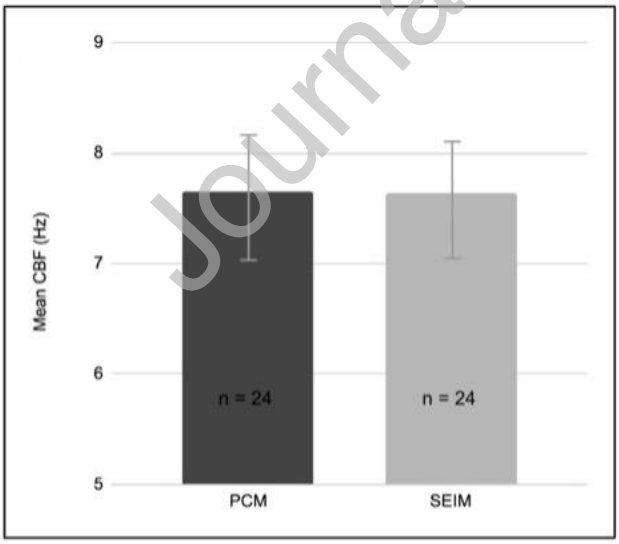

Figure 3

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Figure 3: Mean CBF and standard error for phase contrast microscopy (PCM) (7.65 ± 0.60 Hz) and spectrally encoded interferometric microscopy (SEIM) (7.64 ± 0.51 Hz).

Tables

TABLE 1.
Demographic Features

Characteristic	*N* = 71
Sex *n* (%)	
Female	39 (54.9)
Male	32 (45.1)
Age at surgery	
Median (IQR)	57 (32.5, 64.0)
Range	7.0, 78
Surgery (%)	
Functional endoscopic sinus surgery	26 (36.6)
Endoscopic skull base surgery	20 (28.2)
Rhinoplasty with turbinate reduction	20 (28.2)
Mass resection	5 (7.0)
Phenotype (%)	
Control	45 (63.4)
CRSwNP	11 (15.5)
CRSsNP	15 (21.1)
Endotype (%)	
Control	45 (63.4)
eCRS	7 (9.9)
neCRS	19 (26.7)
Location (%)	
Inferior Turbinate	19 (26.8)
Middle Turbinate	8 (11.2)
Superior Turbinate	2 (2.8)
Ethmoid Sinus	18 (25.4)
Sphenoid Sinus	9 (12.7)
Maxillary Sinus	5 (7.0)
Uncinate Process	9 (12.7)
Lateral nasal wall	1 (1.4)

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TABLE 2.
Ciliary Beat Frequencies

Characteristic	*N* = 71
Phenotype	**Mean CBF (Standard Deviation)**
Control	6.28 (1.95)
CRSwNP	5.77 (1.43)
CRSsNP	6.05 (1.95)
Endotype	
Control	6.28 (1.95)
eCRS	5.74 (1.43)
neCRS	6.00 (1.93)
Location (%)	
Inferior Turbinate	6.15 (1.96)
Middle Turbinate	5.70.39)
Superior Turbinate	5.32 (1.21)
Ethmoid Sinus	5.94 (1.64)
Sphenoid Sinus	5.88 (1.50)
Maxillary Sinus	6.42 (2.21)
Uncinate Process	6.32 (2.04)
Lateral nasal wall	5.15 (2.23)

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