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EARLY DETECTION OF LUNG CANCER USING ARTIFICIAL INTELLIGENCE-ENHANCED OPTICAL NANOSENSING OF CHROMATIN ALTERATIONS IN FIELD CARCINOGENESIS

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ABSTRACT: Supranucleosomal chromatin structure, including chromatin domain conformation, is involved in the regulation of gene expression and its dysregulation has been associated with carcinogenesis. Prior studies have shown that cells in the buccal mucosa carry a molecular signature of lung cancer among the cigarette-smoking population, the phenomenon known as feld carcinogenesis or feld of injury. Thus, we hypothesized that chromatin structural changes in buccal mucosa can be predictive of lung cancer. However, the small size of the chromatin chain (approximately 20 nm) folded into chromatin packing domains, themselves typically below 300 nm in diameter, preclude the detection of alterations in intradomain chromatin conformation using difraction-limited optical microscopy. In this study, we developed an optical spectroscopic statistical nanosensing technique to detect chromatin packing domain changes in buccal mucosa as a lung cancer biomarker: chromatin-sensitive partial wave spectroscopic microscopy (csPWS). Artifcial intelligence (AI) was applied to csPWS measurements of chromatin alterations to enhance diagnostic performance. Our AI-enhanced buccal csPWS nanocytology of 179 patients at two clinical sites distinguished Stage-I lung cancer versus cancer-free controls with an area under the ROC curve (AUC) of 0.92± 0.06 for Site 1 (in-state location) and 0.82 ± 0.11 for Site 2 (out-of-state location).

INTRODUCTION

Cancer screening tests should, ideally, identify cancer before symptoms have appeared and while the tumor is small in order to effectively increase the chance of treatment and reduce mortality. Lung cancer is the leading cause of cancer deaths across races and genders in the U.S. with an overall 5-year survival rate of 22.9% which is notably lower than colorectal (65.1%), breast (90.6%), and prostate cancers (96.8%)1. However, if lung cancer is detected at an early stage it is highly curable through surgical resection. The 5-year survival rate for late-stage (distant) non-small lung cancer (NSLC) is less than 8% but improves to

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64% if detected at a localized stage, and reaches 80% if detected at Stage-IA2. Low-dose computed tomography (LDCT) has been established as the gold standard for lung cancer screening and is associated with a 20% decrease in mortality among patients screened with the technique. Accessibility, cost, stigma, and lack of adherence to LDCT guidelines are among the major challenges limiting its impact, as only about 5% of the LDCT-eligible population undergoes screening3, resulting in 55% of lung cancer cases being detected at an advanced stage where the survival rate is below 8%4. We therefore propose a minimally invasive, accessible, sensitive, and accurate screening test with high sensitivity (Se) to early-stage lung cancer. Screening methods other than LDCT such as chest X-rays and sputum cytology have proven unsatisfactory when evaluated in large-scale clinical screening settings5. New methods based on standard protein biomarkers used for the detection of cancer do not provide sufficient sensitivity and specificity (Sp)6. Recently, there has been significant interest in the development of protocols that rely on tumor secretions in the blood, such as liquid biopsy. Tests being developed by companies including Grail, Freenome, Guardant, Delfi, and Thrive identify cancer by analyzing circulating tumor DNA (ctDNA) or tumor-derived circulating free DNA (cfDNA) properties such as gene mutations, methylation, and fragmentation7,8,9,10,11. Although initial results have shown promise in the detection of various cancers, including lung cancer, the sensitivity to Stage-I and smaller lesions drops precipitously below a clinically acceptable level. It has been suggested that this is not primarily a technological limitation, but may instead be related to the biology of the source and type of biomarker. Smaller lesions secrete less tumor ctDNA (~ 1 ctDNA/ 10 mL of blood), while tumor heterogeneity can only be modeled through many tumor-byproduct biomarkers, which makes it challenging to find the needed quantities of ctDNA in a clinically practical blood sample12. For example, the overall sensitivity of the Grail multi-cancer early detection (MCED) test drops from 90.1% [95% confidence interval (CI) 87.5–92.2%)] in Stage-IV patients to 16.8% [95% CI 14.5–19.5%] in Stage-I patients13. Liquid biopsy can be a powerful tool for non-screenable cancers (pancreatic, etc.) but for cancers with established screening protocols, such as colorectal and lung, methods to detect highly treatable early-stage lesions are still urgently needed. To address these issues and develop an effective screening test for

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lung cancer, we optimized three crucial aspects: (1) biomarker source, (2) biomarker type, and (3) enabling technology. An ideal biomarker source for the development of a large-scale screening test should be obtainable through a minimally invasive procedure, with an easy-to-implement and reproducible protocol, and provide high sensitivity to small treatable lesions14. Our approach to finding this biomarker source relies upon the application of a well-established phenomenon known as field carcinogenesis (or field effect, field of injury) which was first introduced six decades ago15. In field carcinogenesis, the genetic/epigenetic alterations leading to neoplastic cell transformation are distributed diffusely throughout the "field of injury" even at the pre-malignant stage15,16,17,18,19,20,21,22,23. In molecular field carcinogenesis, tumors arise on a histologically normal-appearing, phenotypically silent, but preconditioned and premalignant 'field'. This field carries transcriptomic, genomic, and epigenetic alterations, which can be indicative of an ensuing neoplasm within the affected region20,24.Due to the stochastic nature of these molecular changes, some cells may eventually give rise to a tumor clone. Thus, in lung field carcinogenesis, cells throughout the entire aero-digestive mucosa harbor molecular biomarkers of carcinogenesis regardless of their proximity to a tumor16,17. The buccal mucosa is widely recognized as a "molecular mirror" for lung cancer because of field carcinogenesis16,18,19,25 and we considered it as our biomarker source for two reasons. First, buccal (cheek) brushings are easily performed and uniquely suited for an at-home test or for a primary care office, dentist, etc., as opposed to "liquid biopsies" that can hardly be self-administered. Next, due to the etiological relationship between field carcinogenesis and the rise of tumors on this molecular background, as a biomarker field carcinogenesis is expected to be highly sensitive to early (e.g., Stage-I) cancers, regardless of tumor size, which is diagnostically crucial and an important difference from other sources such as blood or breath which depend on the load of secretions by a tumor, and thus are more sensitive to large tumors than small ones. Determining a suitable lung cancer biomarker type from buccal mucosa is the next major challenge. Biomarkers obtained from genetic changes are negatively impacted by the extremely high number of genetic alterations and astonishing tumor heterogeneity that hampers the application of downstream biomarkers for detection of small lesions. On the other hand, dynamic

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chromatin structure is a regulator of global patterns of gene expression, affecting the binding constants of transcriptional reactants, their diffusion to the sites of transcription, and gene accessibility to the reactants, including transcription factors (TF) and RNA polymerases (RNAPs)26,27. In particular, chromatin structure has been shown to be a regulator of cellular transcriptional plasticity, which is one of the critical etiological hallmarks of carcinogenesis, making it a potential candidate biomarker for early-stage lung cancer detection26,27,28. To understand what types of chromatin structure may foster carcinogenesis, we first needed to calculate a quantifiable metric of chromatin structure. We and others have reported that chromatin is organized as a variety of packing domains29,30,31. At the smallest length scale, DNA wraps around histones and forms ~ 11 nm nucleosome complexes of "beads on a string" which are further folded into the curvilinear chromatin chain, between 5 and 24 nm32. These chromatin chains are packed together in various structural compactions and densities forming irregular blocks of larger packing domains. The packing domains have heterogeneous morphological properties with an average radius of 80 nm and genomic size of about 200 kbp33. Within these domains, chromatin shows a polymeric fractal-like behavior (i.e., the mass scaling behavior within domains follows a near-power-law relationship) along with radially decreasing mass density from the center to the periphery33. Chromatin packing scaling (D) is defined by estimating the number of base Experimentally measured values of D fall between 5/3 and 3 across packing domains33. A higher D value may indicate a packing domain with an increased chromatin heterogeneity and a decreased gene connectivity, resulting in more frequent longer-distance contacts34,35. Chromatin domain structures with a higher D have been linked to further upregulation of initially upregulated genes and concomitant suppression of downregulated genes26,34. In turn, these processes result in transcriptional patterns with greater transcriptional malleability and intercellular transcriptional heterogeneity26,33. As neoplastic cells must keep developing new traits in response to stressors (e.g., hypoxia, immune system attack, new microenvironment, chemotherapy), they benefit from transcriptional plasticity. Tumor cells that can more efficiently upregulate critical pro-survival pathways for a given level of stress through transcriptional malleability and

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heterogeneity have a higher likelihood of attaining a rare transcriptional state that is critical for cancer cell survival, thus further carrying this transcriptional phenotype through replication and increasing the probability of their progeny to acquire other gene mutations, some of which may be beneficial to tumorigenesis. Thus, transcriptional plasticity-facilitating chromatin states (including higher chromatin packing domain may play a critical role in creating a "proneoplastic positive feedback loop" and therefore serve as a marker for neoplastic progression35. A significant correlation between proneoplastic processes with higher packing scaling D, as well as transcriptional plasticity across different malignancies, supports the concept of chromatin-regulated transcriptional plasticity. In particular, a comprehensive analysis of the TCGS (The Cancer Genome Atlas) database revealed that transcriptional divergence in late-stage (Stage-III–IV) tumors at the time of diagnosis is an independent predictor of survival time among patients with lung, colon, and breast cancer26.Chromatin structural changes occur from across the chromatin chain to domains at length scales from ~ 20 to ~ 300 nm, which is too small to be observed by conventional optical microscopy. In order to reproducibly measure these sub-diffractional chromatin alterations, we developed a new technique called chromatin-sensitive partial wave spectroscopic (PWS) microscopy, based on the physical principles of statistical spectroscopic nanosensing. csPWS is a fast, reliable, and nanoscale-sensitive optical spectroscopic technique that can detect chromatin conformation changes with sensitivity between 23 and 334 nm36. The key innovation in csPWS is statistical nanosensing in which sub-diffractional structures, while not resolvable through conventional optical microscopy, are detectable through analysis of the spatial variations of the refractive index (RI) via the spectroscopy of scattered light interference within each of the microscopic resolution voxels25,37,38,39,40,41,42. The output of csPWS microscopy is an image of the cell nucleus where the spectrum resulting from the interference of light scattered by the subdiffractional spatial variations of chromatin density with a reference wave is processed to measure chromatin packing scaling D30,33,43 describes a quantitative statistical measurement of the three-dimensional packing of the chromatin polymer within a self-similar domain. However, local physical conditions such as nuclear crowding density, genomic size

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(Nd), domain volume fraction, and domain intracellular positioning (peripheral vs. interior, etc.) are also important physical regulators that help determine chromatin connectivity, accessibility, and transcriptional plasticity, and therefore, gene expression26,44. As packing scaling D is not the only predictor of plasticity-fostering conformation, calculating average D will not fully capture the complexity of the chromatin regulatory mechanisms influencing gene expression. Thus, we utilized advanced machine learning algorithms and artificial intelligence (AI) to distinguish the biological footprints of lung cancer contained in the images of nuclear D. Such a novel "hybrid" AI + etiological biomarker approach is made possible—and potent—by developing neural network (NN) layers informed with mechanistic data obtained from the chromatin structure alterations contained in the packing scaling D image. In this fashion we coupled our novel csPWS microscopy with a knowledge-based AI approach and achieved high sensitivity for the detection of early-stage lung cancer.

Material and methods csPWS nanocytology involves the collection, shipment, and preparation of buccal samples followed by csPWS image acquisition and evaluation of the nuclear chromatin packing scaling D image using AI enhancement.

Patient recruitment. Patients were recruited through Institutional Review Boards approved at Northwestern University, Northwestern Memorial Hospital, and Boston Medical Center/Boston University. All methods were performed in accordance with the relevant guidelines and regulations and written informed consent was obtained from all participants. Te cohort comprised 96 patients with histologically confrmed lung cancer within 1 year prior to recruitment (case population) and 83 patients with a negative LDCT scan within 1 year prior to recruitment (control populations). 167 patients were over 45 years of age, nine patients were 27 to 44 years of age, and the age of three patients was unknown. Exclusion criteria were family history of lung cancer, exposure to chemotherapy and radiation in the past 3 months, pregnant/lactating women, and inability to give informed consent. Our data were obtained with discovery and independent validation of datasets from Site 1, Northwestern Memorial Hospital (NMH) in Chicago, Illinois, US, and Site 2, Boston Medical Center (BMC) in Boston, Massachusetts,

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US. Te control population included non-smokers, low-risk and high-risk smokers, and patients with benign nodules. Te lung cancer patients included all stages but were predominantly Stage-I patients (62% for Site 1 including 11% Stage-IA, and 76% for Site 2, including 14% Stage-IA).

Sample collection. Buccal samples were collected in the primary care physician's ofce through a buccal swab procedure using a minimally invasive standard of care (Cytobrush, CooperSurgical, Inc., Trumbull, CT, USA). Te patients rinsed their mouths with water three times before the physician placed the bristles on the inside of one buccal surface followed by a top to down motion including brush rotation. Next, the impregnated swabs were dipped into 1.5 ml vial tubes (Neptune Scientifc, San Diego, USA) containing 750 ml of 25% ethanol (collection bufer). Te samples were then packaged and shipped to the central lab for csPWS microscopy.

Sample shipment. Te Site 2 samples were shipped through the air from an out-of-state location while the Site 1 samples were shipped by ground transportation from an in-state location. Te samples were maintained at a temperature below 10 °C during transport using a custom-built transport kit and were received at the central facility within 24 h of sample collection. Te transportation kit included an outer corrugated box (Uline, Pleasant Prairie, WI, USA) and polar pack refrigerants (SONOCO Termosafe, Arlington Heights, IL, USA) and temperature was monitored using a temperature indicator (Timestrip, Cambridge, UK). Te sealed vial was packaged using an inner Styrofoam container and absorbent sheets to avoid possible leakage under refrigerated conditions.

Sample preparation. Clinical samples were prepared within 24 h of collection based on the approaches reported earlier45. In brief, the samples in 25% ethanol were spray deposited on a Fisher brand Superfrost microscope slide (Fisher Scientifc, Hampton, NH, USA) using our custom-built cell deposition system to form a non-overlapping monolayer of buccal cells. Te sample slide was air-dried prior to cytological fxation with 95% ethanol (Termo Fisher Scientifc, Waltham, MA, USA) followed by csPWS microscopy.

Standard operating procedure (SOP). We developed a csPWS SOP to capture buccal nuclear chromatin structural changes. Our goal was to ensure a fast, robust, reliable, and repeatable protocol

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with small variability of physical features of the cells acquired by csPWS from each patient. To minimize the complexity at the collection site, we carried out the cell fxation and sample deposition at the central lab instead of the primary care ofce45. For each patient, a total of>30 cells were collected, where the sample size number was determined by power analysis with the confdence interval (CI) on mean D restricted to less than 5% of the diference between cancer and control population45. We created a sample transport solution of 25% ethanol and used our custombuilt cell deposition device to spray deposit a non-deformed, non-overlapping monolayer of buccal cells with clear nuclear boundaries on the glass slide. An airdrying step enhanced the attachment of cells to the glass, followed by fxation with 95% ethanol and csPWS microscopy. Te csPWS microscope was controlled via custom sofware with a graphical user interface (GUI). Te imaging procedure began by scanning the whole slide using a 10X air objective. A semi-automated slide-map module was developed to rapidly generate a low-magnifcation image by collecting and stitching individual slide region images. Tis assisted a trained user blinded to the diagnostic information in selecting over 30 buccal cells across the entire slide in a timely manner. Our cell screening protocol selected non-folded and non-overlapping cells with clear nucleus boundaries. Te csPWS spectral acquisition was performed with the cells in a liquid medium (95% ethanol) using a liquid-dipping 40X optical objective (Nikon, Melville, NY, USA) to match the RI between the buccal cell and liquid cover Te csPWS acquisition algorithm automatically acquired spectral data for selected cells, and the analysis algorithm rapidly generated the processed spectral data. Tese processes facilitated reliable and reproducible results, making csPWS suitable for larger future studies that include additional clinical sites.

CONCLUSION:

The integration of artificial intelligence (AI) into the early detection of cancer represents a monumental leap forward in modern healthcare. The existing system, bolstered by AI algorithms, has shown remarkable promise in revolutionizing how we identify and address malignancies at their nascent stages. This technology's ability to analyze diverse medical data with precision and efficiency has led to improved accuracy and sensitivity in cancer diagnoses, potentially reducing instances of both false positives and negatives. Furthermore, the proposed system's emphasis on personalized

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treatment plans, factoring in genetic, lifestyle, and environmental influences, holds the potential to significantly enhance patient outcomes. The efficiency gains afforded by AI-driven diagnostics are invaluable, offering timely interventions that can be pivotal in a patient's prognosis. However, it is imperative to approach this advancement with careful consideration of ethical, legal, and regulatory implications. Striking a balance between technological innovation and the expertise of healthcare professionals will be key in realizing the full potential of AI in early cancer detection. With continuous research, development, and collaboration, we stand on the cusp of a new era in oncology, where AI plays an integral role in improving lives and reshaping the landscape of cancer care.

REFERENCES:

1.McPhail S., Johnson S., Greenberg D., Peake M., Rous B. Stage at diagnosis and early mortality from cancer in England. *Br. J. Cancer.* 2015;112:S108–S115. doi: 10.1038/bjc.2015.49. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

2. Knight S.B., Crosbie P.A., Balata H., Chudziak J., Hussell T., Dive C. Progress and prospects of early detection in lung cancer. *Open Biol.* 2017;7:170070. doi: 10.1098/rsob.170070. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

3. National Cancer Registration and Analysis Service: Staging Data in England. [(accessed on 14 October 2021)]. Available online: https://www.cancerdata.nhs.uk/stage_at_diagnosis

4. NHS NHS Long Term Plan: Cancer. [(accessed on 30 March 2021)]. Available online: https://www.longtermplan.nhs.uk/areas-of-work/cancer/

5. Sasieni P. Evaluation of the UK breast screening programmes. *Ann. Oncol.* 2003;14:1206–1208.doi: 10.1093/annonc/mdg325. [PubMed] [CrossRef] [Google Scholar]

6. Maroni R., Massat N.J., Parmar D., Dibden A., Cuzick J., Sasieni P.D., Duffy S.W. A case-control study to evaluate the impact of the breast screening programme on mortality in England. *Br. J. Cancer.* 2020;124:736–743. doi: 10.1038/s41416-020-01163-2. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

7. Esserman L.J. The WISDOM Study: Breaking the deadlock in the breast cancer screening debate. *NPJ Breast Cancer.* 2017;3:34. doi: 10.1038/s41523-017-0035-5. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

8. Dembrower K., Wåhlin E., Liu Y., Salim M., Smith K., Lindholm P., Eklund M., Strand F. Effect of artificial intelligence-based triaging of breast cancer screening mammograms on cancer detection and radiologist workload: A retrospective simulation study. *Lancet Digit. Health.* 2020;2:e468–e474. doi: 10.1016/S2589-7500(20)30185-0. [PubMed] [CrossRef] [Google Scholar]

9. Meystre S.M., Heider P.M., Kim Y., Aruch D.B., Britten C.D. Automatic trial

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Vol-11 Issue-04 Nov 2023

eligibility surveillance based on unstructured clinical data. *Int. J. Med. Inform.* 2019;129:13–19.doi: 10.1016/j.ijmedinf.2019.05.018. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

10. Beck J.T., Rammage M., Jackson G.P., Preininger A.M., Dankwa-Mullan I., Roebuck M.C., Torres A., Holtzen H., Coverdill S.E., Williamson M.P., et al. Artificial Intelligence Tool for Optimizing Eligibility Screening for Clinical Trials in a Large Community Cancer Center. *JCO Clin. Cancer Inform.* 2020;4:50–59. doi: 10.1200/CCI.19.00079. [PubMed] [CrossRef] [Google Scholar].

11. Huang S., Yang J., Fong S., Zhao Q. Artificial intelligence in cancer diagnosis and prognosis: Opportunities and challenges. *Cancer Lett.* 2020;471:61–71. doi: 10.1016/j.canlet.2019.12.007. [PubMed] [CrossRef] [Google Scholar]