The Cole Relaxation Frequency as a Parameter to Identify Cancer in Lung Tissue: Preliminary Animal and ex vivo Patient Studies

Les Bogdanowicz (✉ les.bogdanowicz@gmail.com)

NovaScan, Inc.

Onur Fidaner

NovaScan, Inc.

Donato Ceres

NovaScan, Inc.

Alex Grycuk

NovaScan, Inc.

Davis Demos

Aurora St. Luke's Medical Center

Article

Keywords: lung cancer, Cole Relaxation Frequency, diagnosis

Posted Date: November 15th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1075526/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at JMIR Biomedical Engineering on December 1st, 2021. See the published version at <https://doi.org/10.2196/35346>.

The Cole Relaxation Frequency as a Parameter to Identify Cancer in Lung Tissue: Preliminary Animal and *ex vivo* Patient Studies

Bogdanowicz, L., PhD ^{1,2}, Fidaner, O., PhD ¹, Ceres, D., PhD ¹, Grycuk, A. ¹, Demos, D., MD ³

Affiliations

1. NovaScan, Inc., Chicago IL

2. Department of Biomedical Engineering, University of Illinois at Chicago, Chicago, IL

3. Aurora St. Luke's Medical Center, Milwaukee, WI; Department of Cardiothoracic Surgery, Aurora Healthcare, Milwaukee, WI

Abstract

Lung cancer is the world's leading cause of cancer deaths, and diagnosis remains challenging. Lung cancer starts as small nodules; early and accurate diagnosis allows timely surgical resection of malignant nodules while avoiding unnecessary surgery in patients with benign nodules. The Cole Relaxation Frequency (CRF) is a derived electrical bioimpedance signature, which may be utilized to distinguish cancerous tissues from normal tissues.

Here we show that CRF allows for diagnosis of cancer in human subjects, based on evaluation of 60 specimens obtained from 30 patients. We observed clear discrimination of CRF values in tumor and distant normal tissues, resulting in a high degree of sensitivity (97%) and specificity (87%) in cancer diagnosis. Furthermore, we tested 20 xenograft small animal model specimens, observing a similar separation of CRF values as in the human in-vivo measurements. We also obtained CRF measurements in pressurized and unpressurized lungs by implanting tumors into ex-vivo porcine lungs. CRF measurements align with previous tests in human and small animal models.

Introduction

Background

Lung cancer is the cause of 20% of all cancer-related deaths globally and is the most commonly diagnosed cancer worldwide, representing 13% of all cancer diagnoses. Globally it is estimated that lung cancer accounts for 2 million cases per year and 1.7 million deaths per year. ¹ The economic health burden of lung cancer is estimated to be 1.5% of the world's gross domestic product, approx. 1.2 trillion dollars. ²

The diagnosis of lung cancer begins with detection of pulmonary nodules or masses using chest X-rays and low-dose computed tomography (CT) technologies. Nodules and/or masses appear

in 1 out of every 500 chest X-rays, and only about 40% are cancerous.³ These nodules usually develop in the deep periphery of the lung, requiring navigation to branching airway structures to reach them. The nonsurgical gold standard technique to differentiate benign from malignant pulmonary nodules is either transthoracic needle biopsy or bronchoscopy biopsies.⁴ These techniques report a diagnostic accuracy below 78% and 60%, respectively, with decreasing sensitivity for smaller nodules.^{4,5} Consequently, when small nodules (<1 cm) are detected, they are typically monitored for 3-6 months and are pursued upon growth. More advanced technologies that can detect cancer accurately in small malignant lung nodules can help achieve early diagnosis and prompt treatment, which is critical to lung cancer survival. In recent years low-dose computed tomographic screening was shown to reduce lung-cancer mortality by shifting cancer detection from symptomatic late-stage cancers to early-stage cancers, supported by the National Lung Screening Trial (NLST) and the NELSON trial.⁶ The relative survival rate for cancerous nodules is about 50% after 5 years, rising to 80% if the nodule is first diagnosed at a diameter of 1 cm or less.³ In this paper, the authors propose a novel technology, NoduleScan™ that provides an accurate diagnosis of cancerous lung nodules via an electrical assessment of the tissue. The accuracy of NoduleScan™ will be tested by comparing the outcomes (cancerous versus noncancerous) obtained with the new technology to the results of the pathology report on 3 different tissues: (1) Human lung tumors from 30 patients (human lung testing ex-vivo); (2) A549 human lung tumor xenograft grown on mouse model and noncancerous lung tissue from the same mouse model, tested both in-vivo, to include the effect of perfusion, and ex-vivo (animal testing); and (3) healthy ventilated porcine lungs with A549 cancerous nodules insertion to include the effect of breathing and to demonstrate detection sensitivity (porcine lung testing). We hypothesized that NoduleScan™ will discern cancerous and non-cancerous as was previously shown in breast and skin.⁷⁻⁹

NoduleScan™ Technology

Bioimpedance Modeling for Cancer Detection. The characterization of the bio-electrical properties of tissues is often achieved using electrical circuits models, which attempt to describe in electrical terms the frequency-dependent behavior of ion movement within the extracellular and intracellular media, across cellular membrane, as well as membrane polarization effects. The Cole-Cole impedance model, (Figure 1) is often used to describe the impedance behavior of biological tissue.¹⁰

Figure 1: Equivalent Circuit Model

In this representation, the Constant Phase Element (CPE) is an empirical circuit element purely devised to describe polarization phenomena as a result of system inhomogeneity:

$$Z_{CPE} = \frac{1}{C_\alpha(j\omega)^\alpha} \quad (1)$$

where C_α is a pseudo-capacitance and α is its order ($0 < \alpha < 1$).

The Cole-Cole equation (2) derives in principle from the CPE model specifically when describing impedance behavior of biological tissue as

$$Z_{Cole} = R_\infty + (R_o - R_\infty) || Z_{CPE} = R_\infty + \frac{R_o - R_\infty}{1 + (j\omega\tau)^\alpha} \quad (2)$$

where $\tau = \frac{1}{2\pi f_c}$ is the relaxation constant, $C_\alpha = \frac{\tau^\alpha}{(R_o - R_\infty)}$, and f_c is the Cole Relaxation Frequency (CRF).¹¹

The fundamental parameters (R_o, R_∞ , α , and f_c) can be used to describe and characterize the bioimpedance behavior of a tissue. While these parameters do not necessarily describe the physical mechanisms involved in the impedance measurement, in the literature, R_o and R_∞ have been associated with the extracellular medium and fluids, while α and C_α with membrane polarization and ionic permeability¹²⁻¹⁴. Accordingly, we have found⁸ that only the CRF might be sufficient to discriminate between cancerous and noncancerous tissue in the frequency range from 10^3 Hz to 10^7 Hz, often referred to as the β dispersion region, where cellular membranes have the strongest frequency response. This enhanced dispersion sensitivity of the cellular membrane in the β region might provide the physiological interpretation of why the differential membrane polarization and ionic permeability between healthy and cancerous cells in a given tissue result in distinct impedance signatures, and more particularly of CRF values.¹⁵

Recently, it has been demonstrated in a cohort of $n=373$ patients that a single parameter, CRF, can be used to detect breast cancer with sensitivity and specificity of 95% and 87%, respectively.⁸ Similarly, CRF has been used to identify skin cancer with sensitivity and specificity of 100% and 90%^{7,9}, respectively. In the same way, the CFR parameter will be used in the present study as a biomarker for lung cancer detection, based on impedance spectroscopy in the β frequency range.¹⁵

Device used for Testing. The CRF measurements are obtained using a tetrapolar electrode system as shown in Figure 2. The four electrodes are prepared by electroplating of platinum black (PtBlk), which results in non-polarizable electrode surfaces suitable for bioimpedance measurement of tissue. An electrolytic gel¹⁶ is used to provide an electrical coupling between the electrodes and the tissue. When in contact with the tissue, a spectral impedance measurement is recorded at discrete frequencies (32 frequencies per decade) in the β

frequency region. In this tetrapolar configuration, the two outer electrodes are the stimulating electrodes that drive a current through the tissue by applying a 1Vrms voltage, while the two inner electrodes are used to measure the voltage differential. Amplitude and phase of complex bioelectrical impedance is then calculated from the ratio of the voltage differential to the current. Impedance measurement is repeated for each discrete frequency in the β frequency region. Our testing apparatus is optimized to have the highest fidelity in the $2 \times 10^3 \text{ Hz} - 1 \times 10^7 \text{ Hz}$ frequency range.

Figure 2: Photograph and dimensions of measuring electrodes

Analysis of Impedance Data. The raw impedance data is analyzed through the following steps.

1. Remove parasitic resistance-capacitance artifacts coming from the reactive circuit elements in the probe and the cable.
2. Nyquist path analysis of data for initial determination of cancer
3. Find α and f_c values that result in the highest correlation between the impedance formula in Eq. 1 and the data.
 - If the CRF value (f_c) is in the range of $10^5 \text{ Hz} - 2.1 \times 10^6 \text{ Hz}$ (defined as the cancerous range)⁸ then the scan is characterized as “cancer”, otherwise “no cancer”. If there is no CRF value (f_c) present, then the scan is characterized as “no cancer”, solely based on Nyquist path analysis results.
4. Typically, multiple scans are taken from each sample, at slightly different locations. If at least one scan is cancer, then the sample is characterized as “cancer”. Otherwise, if all scans from a sample are “no cancer” then the sample is characterized as “no cancer”.

As an example of the application of the NoduleScan™ technology, Fig. 3 shows the imaginary component of complex impedance computed for two specimens obtained from a mouse model. Fig. 3 (a) shows impedance response of A549 tumor grown on a mouse, which was confirmed to be “cancer” via pathology. For this sample, our scans detected clear peaks at about $6 \times 10^5 \text{ Hz}$, categorizing it as “cancer”. Fig. 3(b) shows the impedance response of a healthy lung sample extracted from the same mouse, which was confirmed to be “no cancer” via pathology.

For this sample, no peaks could be detected in the cancer range; hence the sample was categorized as “no cancer”. Fig. 4 shows the Nyquist path plots of the same data set in Fig. 3, for data points in the cancer frequency range of 1×10^5 - 2.1×10^6 Hz. The circular pattern observed in the Nyquist path plot of tumor samples is the basis for initial cancer/no cancer classification in Step 2.

Figure 3: Sample spectral impedance measurements obtained from mouse model tests. The plots show the imaginary component of measured impedance following removal of parasitic capacitance components. Multiple curves represent repeated measurements of the same tissue in slightly different locations. (a) A549 tumor extracted from mouse (b) normal lung tissue

Figure 4: Nyquist path plot, which shows the imaginary part of measured impedance vs the real part for data obtained from mouse model tests, for frequencies in the cancer range 1×10^5 Hz - 2.1×10^5 Hz. Cancer scans tend to have a concave arch shape, as suggested by the Cole function (Eq.1).

Methods

Human Lung Testing *ex vivo*

Patients (n=30) were recruited from the partner Aurora St. Luke's Medical Center (Milwaukee, WI, USA) under the approved IRB (WCG IRB study number 1264221). Research subjects were recruited from patients, at any site within the Aurora Healthcare system, who had a diagnosis of non small cell lung carcinoma (NSCLC) with tumor size > 1.4 cm, and were elected for a surgical wedge, segmentomy, or lobectomy procedure with or without lymph node dissection.

Study population: Adult men and women presenting with a diagnosis of NSCLC who were candidates for wedge resection or anatomic resection, or thoracic nodal dissection.

Inclusion Criteria: Adult male and female patients, 18 years of age or greater, with a biopsy proven diagnosis of lung cancer and who are scheduled for a wedge resection or anatomic resection. Pre-operative diagnosis is NSCLC that may include the following: invasive carcinoma, squamous cell carcinoma, adenocarcinoma, for those patients scheduled for surgical excision of tissue.

Exclusion Criteria: Patients who refuse consent for study of their excised tissue, patients with a diagnosis of Small Cell Lung Cancer (SCLC), patients with a diagnosis of NSCLC who underwent induction therapy with either chemotherapy or radiation, and patients who are not candidates for surgical resection.

Research subjects were consented preoperatively by the surgeon and Aurora research coordinator. The patient then underwent a lobectomy or segmentomy as per standard of care, and the excisions were bisected. The authors collaborated with the pathology staff to select tissue from two sites for each patient: a piece from the suspected cancerous region and a piece from noncancerous tissue at least 5 cm away from the suspected tumor site. CRF measurements were performed by placing the electrode in different positions on each tissue sample (tumor and away-from-tumor). Once spectral impedance scanning was completed (multiple scans for both tumor and away-from-tumor pieces), the tissue samples were returned to the pathology staff for immediate processing and histological evaluation.

Animal testing

With encouraging results from Human ex-vivo Lung Assessment we pursued answering questions on the ability to discern CA and NC in perfused tissue. Twenty mice were inoculated with a NSCLC line and all survived to full tumor growth. CRF measurements were taken on tumors through skin with a live animal, with the tumor exposed after euthanizing the animal and subsequently the tumor itself was excised and measured. These results were compared to measurements on the excised healthy lung tissue from the same mouse.

Bagg Albino inbred nude mice BALB/c NU/NU (n=25) were implanted with tumor cells that were grown in culture (A549 lung sarcoma cells obtained from American Type Culture Collection, ATCC: CCL-185). These A549 cells (2×10^6 cells in $\sim 100 \mu\text{L}$ sterile PBS) were injected subcutaneously into the right flank of each mouse.

Animals with tumor sizes between 0.1 cm^3 to 1.0 cm^3 were utilized in this study. Tumor measurements were monitored twice weekly until termination. Tumor size was calculated using the formula: $\text{volume} = \frac{1}{2} \text{Length} \times \text{width}^2$.

Spectral Impedance Measurements:

1. **Impedance measurements on tumors with skin intact on live mice:** CRF was measured on live mice on the surface of the intact skin at the tumor site. The live mice were held steady by the nape of their neck and tail and, holding the electrodes in place, measurements were taken on the tumor site. Upon completion of this test, the mice were euthanized.
2. **Impedance measurements on dead mice with exposed tumor:** the tumor site was exposed by cutting a flap from the posterior end of the mouse, extending anteriorly until the entirety of the tumor was accessible. The measurement electrode was then placed directly on the exposed tumor and multiple spectral impedance scans were recorded.

3. **Impedance measurements on ex-vivo tumor:** the tumor was excised in its entirety from the animal and bisected. The sliced faces of the tumor pieces were then placed on the measurement electrode one by one and spectral impedance scans were taken from each piece. One piece was sent to histology for evaluation.
4. **Impedance measurements on noncancerous excised mice lung:** the lungs (noncancerous tissue) were excised from the animal and sliced so that the interior faces were exposed. Spectral impedance measurement scans were recorded. One piece was sent to histology for evaluation.

Porcine Lung Testing

Two healthy porcine lungs were received within 24-26 hours of euthanization with the trachea and vocal cords intact and shipped to the research facility overnight at a constant temperature of 4 °C. The vocal cords were cut out and a manual ventilator was attached and secured tightly in place. The lungs were inflated and deflated using the ventilator and the applied pressure was measured using a gauge.

Separately, five human lung tumors (A549 Cell line) were grown in five separate mice following the procedure in the murine xenograft cancer method discussed earlier. Following tumor inoculation in mice, tumor size was monitored regularly. Once tumor size exceeded 500 mm³, the mouse was euthanized and tumor was excised and bisected. Consequently, each bisected piece size was greater than 250 mm³ on average and ready to be inserted in the porcine lung tissue in order to undergo spectral measurements during simulated breathing

Spectral Impedance Measurements:

1. **Impedance measurements on ex-vivo tumor:** Spectral impedance measurements were recorded on A549 tumors, immediately after excision and bisection. The measurements were done ex vivo on the inner bisected surface of the tumors. One bisected piece was placed in 10% formalin and sent to a histology laboratory for evaluation.
2. **Impedance measurements on healthy porcine lung tissue:** Spectral impedance measurements were performed on porcine lungs when the lung was deflated. Subsequently, the lung was pressurized to 20 mmHg and kept at the inflated state with constant pressure. Lastly, air was slowly released to deflate the lung, at a rate of <1 mmHg per second. During the deflation process, multiple spectral impedance scans were performed.
3. **Impedance measurements on porcine lung with inserted tumor:** A flap was created on the lung to expose the underlying sub-surface lung tissue, by cutting the outermost pleural layer of the lung. The flap tissue was cut to a width that matched the size of the A549 tumor previously excised from the mouse in order to ensure the tumor fit in the lung sub-surface. The thickness of the flap was measured and recorded using a caliper; the target cut tissue thickness was <300 microns. The bisected A549 tumors were

placed in the created lung sub-surface with the flap covering the tumor. Measurements were taken on the surface of the lung at the tumor location with the lungs in the inflated and deflated state. This was repeated for each of the tumor samples. Saline solution was applied on the lungs every 15 min during the studies to maintain tissue hydration.

Results

Human Lung Testing Outcomes

Patients' demographic and tumor characteristics are presented in Table 1 below.

TABLE 1

	No. of patients
Patient age group = 54-85	30
Males	16
Females	14
Adenocarcinoma	21
Squamous Cell Carcinoma	9
pN0	27
pN2	3
ypT0	1
pT1a	0
pT1b	7
pT1c	7
mpT1c	1
pT2	1
pT2a	4
mpT2a	1
pT2b	3
pT3	3
pT4	2

Maximum tumor size presented was 7.5 cm. Per the protocol, subjects with tumor size larger than 1.4 cm were included in the study. The mean size of the tumors was 3.22 cm and median 3.0 cm with a standard deviation of 1.47 cm. Cancerous region for CRF values (1×10^5 Hz – 2.1×10^6 Hz) were compared to the pathology report for cancerous tissue. One tumor sample (confirmed to be “cancer” via pathology) did not yield a CRF value in the cancerous frequency range, resulting in a false negative. We found CRF values in the cancerous range for three ‘normal’ tissue specimens (confirmed to be “no cancer” via pathology) that were at least 5 cm away from the tumor lesion, resulting in false positives. Overall sensitivity and specificity values (combined tumor and distant tumor tissue) are 97% and 87%, respectively. The positive predictive value (PPV) is 88% and the negative predictive value (NPV) is 96%. Results are summarized in Table 2.

Figure. 5: Histogram of CRF measurements from human lung samples. Top plot shows the results for cancerous tumor samples, as determined by pathology. Bottom plot shows the results for measurements determined to be healthy tissue by the pathologist. Note that multiple scans were taken per sample. When cancer scans are present, a sample is categorized as “cancer” and below threshold scans are ignored. The data points in the histogram represent the log-mean of measured CRF values.

TABLE 2

		Pathology Outcome Tumor Tissue		Pathology Outcome Distant Tissue (normal)		Pathology Outcome Overall		
		Cancer	No Cancer	Cancer	No Cancer	Cancer	No Cancer	
CRF DX	Cancer	28	0	1	4	29	4	PPV: 88%
	No Cancer	1	1	0	25	1	26	NPV: 96%
						Sensitivity: 97%	Specificity: 87%	

Mouse Model Testing

Tumor Growth. Tumor height and diameter were measured for each mouse in regular time intervals following inoculation. The eventual tumor sizes had a mean of 432 mm³ and standard deviation of 222 mm³.

Impedance measurements on tumors with skin intact on live mice. CRF peaks were detected in the cancer range for all confirmed as tumors from the pathology report. The median CRF value was determined to be 3.11×10^5 Hz. In some samples multiple distinct CRF peaks can be observed, a phenomenon reported earlier in the literature,⁸ In tumor samples, we typically observed a primary CRF in the higher frequency range, the cancerous range, and a secondary CRF value in the lower frequency range. These secondary CRF values correspond to noncancerous tissue. The presence of noncancerous CRF measurements and cancerous CRF measurements reflect the heterogeneity of lung cancer.¹⁷

Impedance measurements on dead mice with exposed tumor. Impedance measurements recorded on various spots on the exposed tumor. The median CRF value was determined to be 4.36×10^5 Hz.

Impedance measurements on extracted tumor. Measurements of the sliced tumor were similar to those of the exposed tumor. The median CRF value of sliced tumors was determined to be 8.32×10^5 Hz. All histology data confirmed that the tissue pieces were cancerous. This offers a direct comparison of the CRF curves for tumor and no-tumor cases. A comparison of CRF frequency histograms for tumor slices and lung slices is provided in Figure 6 below.

Figure 6: Histogram of CRF measurements of A549 tumors grown on mice. The horizontal axis represents the base 10 logarithm of frequency (Hz) values. The histograms are formed using CRF values from live mouse, exposed tumor, extracted tumor, and extracted lung (healthy tissue). Typically, 3-4 scans taken from each sample. When cancer scans are present a sample is categorized as “cancer” and below threshold scans are ignored. The data points in the histogram represent the log-mean of measured CRF values.

The analysis reveals that all the CRF measurements for lung slices (no tumor sample) are below the decision threshold of $1 \times 10^5 \text{ Hz}$, with a median CRF frequency of $9.68 \times 10^3 \text{ Hz}$. On the other hand, most of the CRF peaks for tumor slices samples are above the decision threshold, with a median CRF frequency of $8.28 \times 10^5 \text{ Hz}$.

In Table 3 we show the comparison of pathologist outcome of tumor samples compared to CRF scans and healthy lung tissue compared to CRF measurements. For the 10 samples analyzed, the outcomes corresponded to 100% sensitivity and specificity.

TABLE 3 Sensitivity and Specificity of Mouse Model Testing

		Pathology Outcome Tumor Tissue		Normal Lung Tissue (No Cancer)		Pathology Outcome Overall		
		Cancer	No Cancer	Cancer	No Cancer	Cancer	No Cancer	
CRF DX	Cancer	10	0	0	0	10	0	PPV: 100%
	No Cancer	0	0	0	10	0	10	NPV: 100%
						Sensitivity: 100%	Specificity: 100%	

Porcine lung testing

Impact of Simulated Breathing on CRF Values: To understand the impact of simulated breathing, in Fig. 7, we compare the histograms for deflated, inflated, and during simulated breathing cases. Data include short and long tether measurements (color coded) for subsurface (electrode on flap) and subsurface (electrode on tumor) cases combined. A no CRF peak measured is classified as non-cancer. There is a smaller amount of CRF measurements in the no cancer range resulting in limited information in histogram plots.

Figure 7: Histograms of CRF data for A. Tumor Only B. Deflated C. Inflated D. During Simulated Breathing cases. There is a shift in the log-mean of CRF peak frequencies among simulated breathing scenarios (deflated/inflated/during) and is within 0.05 log cycles.

Conclusions

We set out to test a novel technology, NoduleScan™, to provide a diagnosis of cancerous lung nodules via an electrical assessment of the tissue. Further we have shown that the diagnosis of CA and NC tissue is in line with those seen in breast tissue and skin tissue.⁷⁻⁹

We have shown that ex-vivo CRF measurements of excised human lung cancerous nodules can differentiate cancerous and noncancerous tumors with a sensitivity of 97% and specificity of 87%.

In xenograft mouse models, tumor growth was found to be in agreement with published literature on mice inoculated with the same amount of A549 cells.¹⁸ A549 lung sarcoma tumors

grown in mice exhibit the same distinction in CRF values as ex-vivo lung tissues and those of previously reported breast and skin tissue. The measurements remain the same when measured in vivo through the skin, with the skin opened tumor exposed and tumor excised. There was no difference in CRF measurements with the tumor perfused or not perfused. When the tumor measurements, confirmed to be cancerous by a pathological examination, were compared to the healthy lung tissue of the sacrificed mouse, the sensitivity and specificity were 100%.

Clear separation of CRF distributions, as shown in Figure 6, demonstrates that CRF is a viable parameter to identify the presence of cancer. Cancer determination is done through multiple scans from the same tissue sample. For each of these below-threshold primary peaks, there was at least one other scan from the same tissue that exhibited cancer-range CRF frequency, hence correctly classifying the sample as “cancer”.

CRF values could be detected and remained relatively unchanged during simulated breathing. CRF values are in the same ranges as shown in ex-vivo human tumor tests, xenograft mice and previously demonstrated breast and skin tissues.

Discussion

Patients of interest. Table 2 shows a pathological assessment of a suspect lesion as non-cancerous, and secondarily a normal tissue assessment as cancerous. These patients were: patient #6036 a 60 y.o. female undergoing a Right Upper Lobectomy and study patient #6086 a 72 y.o. female undergoing a right lower lobectomy. For patient 6036, the nodule presented did not show any CRF values in the cancerous range, and this was later confirmed by pathological examination as fibrotic tissue, TNM status ypT0, pN0 and no cancer was detected showing concordance with the scanned results. Patient 6086 measured CRF values in the cancerous range for tissue distant from the tumor (>5cm,) and pathological examination confirmed positive margins and thus showed concordance with our measurements.

NoduleScan™ technology. The NoduleScan™ assessment is completed rapidly (seconds to make a full scan.) This can be completed without expensive or bulky capital equipment. Further, learning to interpret CRF measurements, while the physician does need to interpret, is straightforward and not nearly as subjective as interpreting confocal microscopy measurements, for example.

As a surgical tool for assessing clear margins. The aim of surgical therapy for the treatment of lung cancer is to excise malignant tumors with clear margins meaning cancer cells are not present at the divided edge of the tissue that was removed.¹⁹ The definition of an adequate margin width is loosely defined as 2 cm or the same as the diameter of the tumor. The tenet that a positive margin (cancer cells present at the cut edge of the excised tissue) results in an increased incidence of cancer recurrence is widely accepted. A “positive” margin is, in a sense, a surrogate for residual tumor and those patients have been shown to have an increased risk of local recurrence and mortality.^{19–21}

Pathology evaluation of margins involves sampling the edges of tissue from all cut faces of the excised specimen. It's impractical for a pathologist to microscopically examine the entire margin in a specimen. The technology presented allows for electrodes to be embedded on a hand held or robotic surgical tool allowing the surgeon to assess the margin quickly and effectively in the operating theater. In circumstances of a positive margin, adjuvant therapy can be implemented to help eliminate residual local disease.²⁰ Classification of tumors as microscopically positive (R1), versus tumors with margins grossly involved by tumor (R2) could be impacted by such a point-of-care analysis of a specimen.^{20,22-27}

As a tool for collecting efficient biopsies. Non diagnostic rates for biopsies in CT guided needle biopsies is reported to be 27.6%. Nodules presented smaller than 1 cm have a 40% non-diagnostic rate.²⁸ One out of four biopsies are inefficient, exposing the patient to complications and risk. The electrodes in the system demonstrated herein can be miniaturized and can fit within a 2 mm orifice of the bronchoscope or on the surface of a biopsy needle. Being able to have a device that can give an indication of malignancy without having to directly sample the tissue would give this process a much higher yield as some of those nondiagnostic samples could also come with a CRF that demonstrates the nodule is malignant. The electrodes can be spaced to assess the entirety of a nodule allowing to compensate for the heterogeneity of the nodule. The measurement can provide an accurate, real-time answer on the presence of cancer. Lesions that were previously difficult to locate from the airway in real time can be easily located as the technology can visualize macroscopically from the airway and identify both cancerous and benign nodules. This provides a precise periprocedural intralesional assessment mechanism to confirm location of cancerous cells within a lesion.

Lymph node assessment. Lymph node status can often influence the decision to proceed with surgery first or induction chemotherapy (N2 nodes). Further, an N3 lymph node will influence the decision of whether or not surgical resection is indicated at all. These nodes can be assessed either by mediastinoscopy before a planned resection, or during the planned resection but before the resection itself has taken place. Real-time nodal assessment with elimination of the time and resource intensive frozen pathologic analytical process and its inherent uncertainties would be invaluable in these circumstances.

Residual Tumor. There are situations where a patient receives induction chemotherapy and/or radiation therapy followed by surgical resection. This involves 5-10% of lung cancer patients, but a much larger proportion of esophageal cancer patients. Sometimes their preoperative induction therapy results in complete pathologic response (CPR), in which no viable tumor is detected on the pathologic evaluation. Measuring a CRF in this patient population could potentially significantly impact this assessment. In the setting of a CPR, a positive CRF might put that designation into question and have some implications as to recurrence risk. Similarly, in patients who are treated with definitive chemoradiation therapy (CRT) who are not surgical candidates and are under post-treatment surveillance, ability to assess the treatment area with a CRF could potentially lead to earlier detection of recurrence. Furthermore, this could prove valuable if a local endoscopic resection margin is uncertain.

References

1. Bray, F. *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* **68**, 394–424 (2018).
2. American Cancer Association. THE GLOBAL ECONOMIC COST OF CANCER. (2010).
3. Pulmonary Nodules - Health Encyclopedia - University of Rochester Medical Center. <https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=22&contentid=pulmonarynodules>.
4. Harzheim, D., Eberhardt, R., Hoffmann, H. & Herth, F. J. F. The solitary pulmonary nodule. *Respiration* **90**, 160–172 (2015).
5. Bubendorf, L., Lantuejoul, S., de Langen, A. J. & Thunnissen, E. Nonsmall cell lung carcinoma: Diagnostic difficulties in small biopsies and cytological specimens. *European Respiratory Review* **26**, (2017).
6. de Koning, H. J. *et al.* Reduced Lung-Cancer Mortality with Volume CT Screening in a Randomized Trial. *New England Journal of Medicine* **382**, 503–513 (2020).
7. Shell, J. & Gregory, W. D. Efficient cancer detection using multiple neural networks. *IEEE Journal of Translational Engineering in Health and Medicine* **5**, (2017).
8. Gregory, W. D. *et al.* The Cole relaxation frequency as a parameter to identify cancer in breast tissue. *Medical Physics* **39**, 4167–4174 (2012).
9. Svoboda, R. M., Gharia, M. J., Shell, J. & Gregory, W. D. Bioimpedance measurement as an assessment of margin positivity in Mohs surgical specimens of nonmelanoma skin cancer: Management implications. *Journal of the American Academy of Dermatology* **79**, 591–593 (2018).
10. McAdams, E. T. & Jossinet, J. Tissue impedance: A historical overview. *Physiological Measurement* **16**, (1995).
11. Cole, K. S. & Cole, R. H. Dispersion and absorption in dielectrics I. Alternating current characteristics. *The Journal of Chemical Physics* **9**, 341–351 (1941).
12. Freeborn, T. J., Maundy, B. & Elwakil, A. Numerical extraction of Cole-Cole impedance parameters from step response. *Nonlinear Theory and Its Applications, IEICE* **2**, 548–561 (2011).
13. Freeborn, T. J. & Fu, B. Fatigue-induced cole electrical impedance model changes of biceps tissue bioimpedance. *Fractal and Fractional* **2**, 1–13 (2018).
14. Tang, C. *et al.* Modeling the frequency dependence of the electrical properties of the live human skull. *Physiological Measurement* **30**, 1293–1301 (2009).
15. Schwan, H. P. Electrical Properties of Tissue and Cell Suspensions. in *Advances in Biological and Medical Physics* 147–209 (1957).
16. SignaGel Electrode Gel: Parker Laboratories, Inc. <https://www.parkerlabs.com/signagel.asp>.
17. de Sousa, V. M. L. & Carvalho, L. Heterogeneity in Lung Cancer. *Pathobiology* **85**, 96–107 (2018).

18. Nakajima, T. *et al.* Orthotopic Lung Cancer Murine Model by Nonoperative Transbronchial Approach. *The Annals of Thoracic Surgery* **97**, 1771–1775 (2014).
19. Owen, R. M. *et al.* Routine intraoperative frozen section analysis of bronchial margins is of limited utility in lung cancer resection. *The Annals of thoracic surgery* **95**, 1859–1866 (2013).
20. Predina, J. D., Keating, J., Patel, N., Nims, S. & Singhal, S. Clinical implications of positive margins following non-small cell lung cancer surgery. *Journal of Surgical Oncology* **113**, 264–269 (2016).
21. Hancock, J. G. *et al.* Impact of adjuvant treatment for microscopic residual disease after non-small cell lung cancer surgery. *The Annals of thoracic surgery* **99**, 406–413 (2015).
22. He, P., Yao, G., Guan, Y., Lin, Y. & He, J. Diagnosis of lung adenocarcinoma in situ and minimally invasive adenocarcinoma from intraoperative frozen sections: an analysis of 136 cases. *Journal of clinical pathology* **69**, 1076–1080 (2016).
23. Emerson, L. L. & Layfield, L. J. Solitary peripheral pulmonary papilloma evaluation on frozen section: a potential pitfall for the pathologist. *Pathology, research and practice* **208**, 726–729 (2012).
24. Marchevsky, A. M. *et al.* Frozen section diagnoses of small pulmonary nodules: accuracy and clinical implications. *The Annals of thoracic surgery* **78**, 1755–1759 (2004).
25. Gupta, R., McKenna, R. & Marchevsky, A. M. Lessons learned from mistakes and deferrals in the frozen section diagnosis of bronchioloalveolar carcinoma and well-differentiated pulmonary adenocarcinoma: an evidence-based pathology approach. *American journal of clinical pathology* **130**, 11–20 (2008).
26. Sawabata, N. *et al.* Optimal distance of malignant negative margin in excision of nonsmall cell lung cancer: a multicenter prospective study. *The Annals of thoracic surgery* **77**, 415–420 (2004).
27. Gupta, R., McKenna, R. & Marchevsky, A. M. Lessons learned from mistakes and deferrals in the frozen section diagnosis of bronchioloalveolar carcinoma and well-differentiated pulmonary adenocarcinoma: An evidence-based pathology approach. *American Journal of Clinical Pathology* **130**, 11–20 (2008).
28. Elicker, B. M. How Should We Manage Nondiagnostic Lung Biopsies? *Radiology* **290**, 824–825 (2019).