In Vitro Screening with Holographic Incoherent Quantitative Phase Imaging Focuses on Finding Medicaments for Repurposing as Anti-Metastatic Agents Designated as Migrastatics

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Abstract: Live lung cancer cells in vitro were exposed to selected medicaments with putative anti-metastatic potential and examined by time-lapse hiQPI, providing simultaneous measurements of the effect on cell growth and motility with unprecedented accuracy.

1. Holographic Incoherent Quantitative Phase Imaging in repurposing medicaments as migrastatics

It has already been shown that the difference between benign and malignant tumors lies in the absence or presence of invasive or metastatic spread of cancer cells. Malignant spread begins with the invasion of the tumor and continues with the formation of distant metastases. However, current anticancer therapies lack a specific category of anti-invasive and anti-metastatic drugs. Here we use the term "migrastatics" for drugs that interfere with all forms of invasion and metastasis of cancer cells, and to distinguish this class from conventional cytostatics, which are mainly directed against cell proliferation [1].

The process of discovering migrastatics and their contraindications can be efficiently supported by dynamic time-lapse analysis of living cancer cells using Holographic Incoherent Quantitative Phase Imaging (hiQPI) obtained by Coherence Controlled Holographic Microscopy (CCHM) and evaluation of their migratory behavior. Other standard microscopy techniques, such as phase contrast, require long observation times and the images are difficult to quantify. We present a methodology that can rapidly evaluate the viability of tumor cells and evaluate the effects of migrastatic treatment using CCHM.

The CCHM is based on an off-axis setting with an incoherent light source. In contrast with the laser, the incoherent CCHM source enables high-quality hiQPI without specles and parasitic interferences. Thanks to the off-axis setting, one hologram is required for image reconstruction and fast processes can be observed. This method provides high contrast; phase images allow the most reliable and accurate automatic segmentation of cells and monitor morphological and positional changes over time [3]. Therefore, this microscope can easily transform cell properties and dynamics into reliable data for analysis.

The following three potential migrastatics, vincristine (VIN), doxycycline (DOXY), and 4-hydroxyacetophenone (4HAP), were selected for the initial investigation of their effect on cell migration. Vincristine represents a chemotherapeutic drug used to treat many types of cancers. VIN works in part by binding to tubulin protein and stopping the polymerization of tubulin dimers to form microtubules, making the cell unable to separate its chromosomes during metaphase [5], such a cell then can undergo apoptosis [6]. DOXY is a second-generation tetracycline antibiotic used to treat various infections and attenuates the migration of melanoma cells by the focal adhesion kinase (FAK) inhibitory signaling pathway [7]. Both of these drugs were chosen because they are already approved for other applications, and therefore the journey to patients would be much faster. 4-hydroxyacetophenone is a simple molecule that inhibits the contractility, adhesion, invasion, and migration of cancer cells and reduces metastatic burden in an in vivo model of cancer metastasis. This treatment of 4HAP activates nonmuscular myosin-2C to alter actin organization, inhibiting the mechanical program of metastasis [8].

All three putative migrastatics, VIN (100 nM), DOXY (1 mg ml^{-1}), and 4HAP (4 μM) were tested with the adenocarcinoma human lung alveolar basal epithelial cells, A549, using Ibidi μ-Slide $VI^{0.4}$ for 20 hours by CCHM. Cells were cultivated at 37°C in a humidified incubator with 3.5% CO_2 in standard Eagle MEM medium with 10% fetal bovine serum, 20 μM gentamicin and 200 mM L-glutamine. For the time-lapse recording, the medium was enriched with 20 mM TES to maintain pH 7.4. Temperature of 37°C was maintained in the environmental enclosure of the CCHM. The microscope is equipped with a motorized stage enabling multi-field time-lapse recording with the 6 channels of the Ibidi μ-Slide.

Examples of images of A549 cells taken from the 20-hour time-lapse recording using CCHM are shown in Fig. 1. Intensity values in the images measured as phase values were converted to cell dry mass density in pg μm^{-2} and their distribution represents cellular morphology with nanometer accuracy.

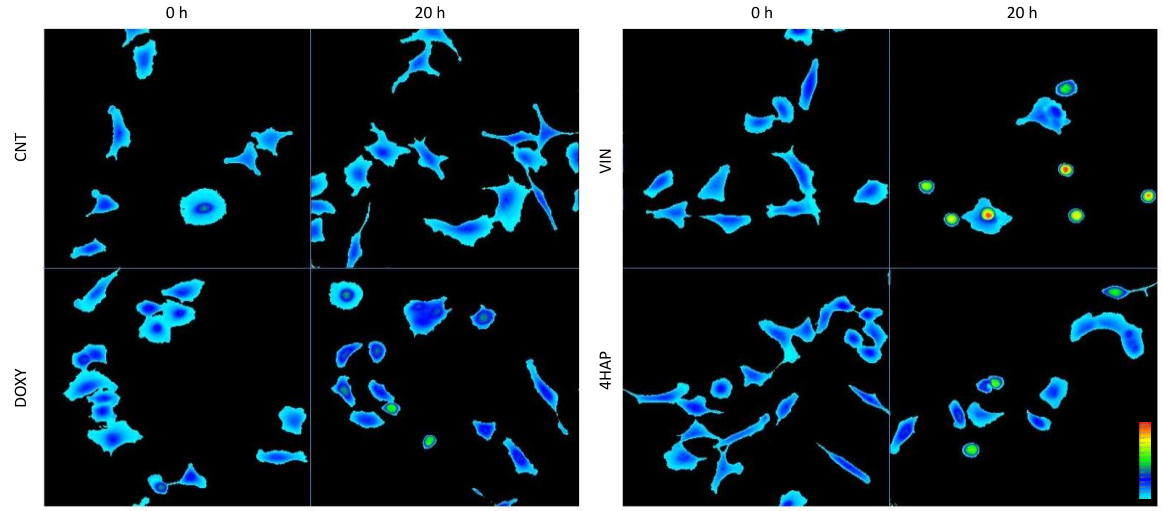

Fig. 1. Example processed images taken from 20-hour multifield time-lapse CCHM recordings with the A549 cells. The potential migrastatics were added before the start. In the control sample, the cells divided, as well as after application of 4HAP and DOXY. In contrast, VIN interfered with cell division. Lapse-interval was 5 minutes. Objective lens was 10× NA 0.3. Field size is 376 µm × 376 µm. Pseudocolor scale represents dry-mass density form 0 (cyan) to 2.1 pg µm^{-1} (red).

2. Pilot testing of candidate medicaments: results and discussion

The main advantage of migrastatic treatment will be the reduction of the most dangerous ability of cancer cells, namely their local penetration (invasion) into the environment and metastasis. In conventional chemotherapy, cancer cells are exposed to stress. However, some of them will survive this stress and, in addition, gain much greater resilience. At the same time, cells begin to leave the original tumor and metastasize. The qualitative advantages of migrastatic treatment over the standard therapies used so far are that tumor cells do not have to be exposed to cytotoxic stress during migrastatic treatment; they are only "kept in place". Thus, the pressure to develop resistance of tumor cells is not as high, and even if resistance to migrastatics develops, it does not confer an advantage on the cells in terms of growth and division - it will not lead to enrichment or even predominance of resistant cells in the tumor [9].

The CCHM defines the pattern of cell polarity by evaluating the morphological profile of migrating cells along with measuring their distribution in the nucleus and peripheral dry-mass. From the hiQPI recordings, we evaluated mean mass doubling time ± S.E.M. which was 29.2 ± 1.8 hours for the control cells and the drugs significantly reduced cell growth resulting in increased mass doubling time to 47.0 ± 3.6 (ANOVA-P < 0.001), 35.7 ± 2.5 (ANOVA P > 0.05) and 41.9 ± 3.8 (ANOVA P > 0.05) for VIN, DOXY and 4HAP respectively.

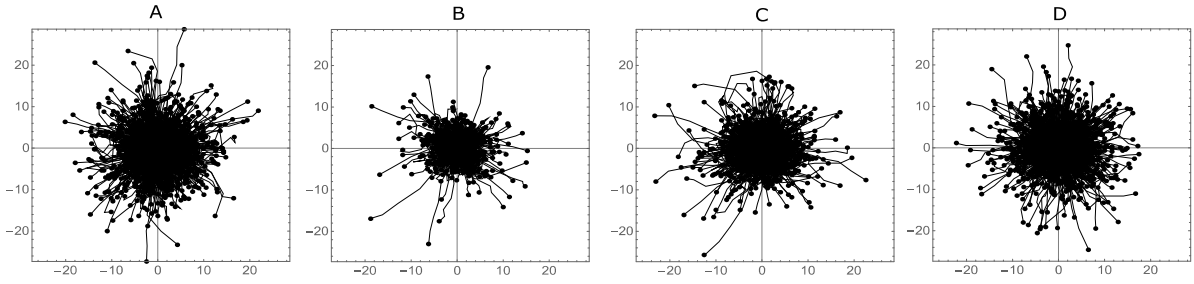

Fig. 2. Rose plots of cell trajectories from hiQPI recordings. Individual 150-min tracks were shifted to the common origin. Axis represent distances in µm. **A)** CNT (n = 3312), **B)** VIN (n = 5386), **C)** DOXY (n = 2989) and **D)** 4HAP (n = 4557).

Motility rose plots illustrate that VIN reduced motility of cells, as their trajectories were short (Fig. 2.). VIN affected speed and also spreading, elongation, and circularity (Fig. 3.) of cells, the most of all three selected drugs. The cells responded the least to 4HAP. DOXY caused changes in cell behavior, but not as dramatic as VIN.

This research showed that in the cancer cell line, VIN had the greatest migrastatic effect in the 2D environment under given conditions.

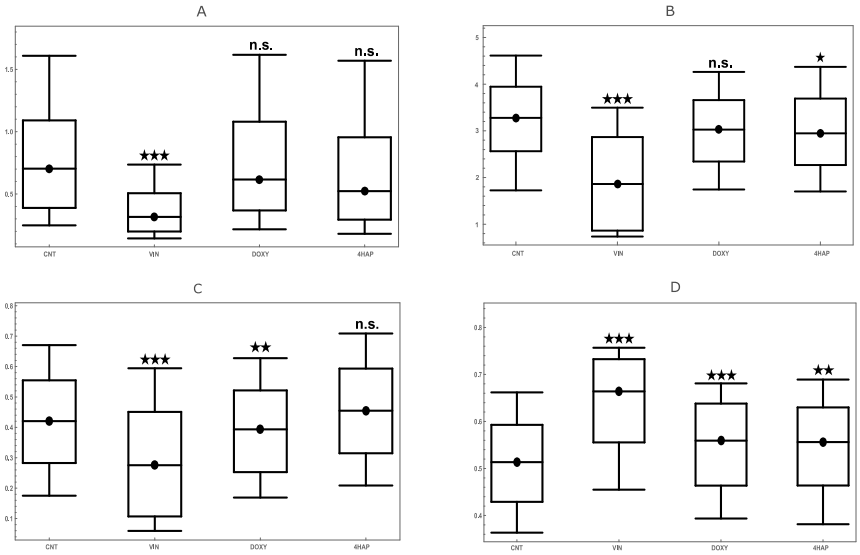

Fig. 3. Box and whisker plots illustrating responses of A549 cells to selected drugs. Dots represent medians, boxes span 50% of data and whiskers 80% of data. **A)** Speed of cell translocation in μm h^{-1}. **B)** Spreading in μm^2 pg^{-1}. **C)** Elongation and **D)** Circularity was calculated in Mathematica using ComponentMeasurements function. * represents ANOVA-P < 0.05, ** ANOVA-P < 0.01, *** ANOVA-P < 0.001 and n. s. if for not significant.

The main idea of this project is to prove that hiQPI is a reliable and economical approach to in vitro testing of potential migrastatics so that they can be considered for the use in clinics. The hiQPI is a realistic candidate for this role because it combines the high precision of cell imaging, which is important for the segmentation of cells and therefore tracking cell trajectories, with measuring cell growth and thus providing a complex evaluation of the effects of the migrastatic treatment. This avoids the possible error of other imaging techniques, which rely on detecting migration suppression only, while growth is not accurately evaluated. We intend to introduce the hiQPI and upgrade it to a standard tool. The most ambitious aspect of the project is the highly quantitative analysis of cell motility as related to growth at the individual cell level measuring the intrinsic intra-tumor heterogeneity of cancer cells. The critical aspect will then be the discovery of migrastatic influence on various cohorts within the intrinsic heterogeneity of cancer cells. Particularly, the identification of suppression of a highly motile fraction, which we will directly measure, can have a profound clinical impact. This is because single-cell analysis is an essential aspect of our approach and is complementary to cell population studies, such as microarray profiling, which can be better focused on dangerous cells. Profit from this project is of great interest to doctors and the basics of cancer treatment.

3. Acknowledgments

This research was supported by the Czech-Bioimaging: National Infrastructure for Biological and Medical Imaging (LM2018129).

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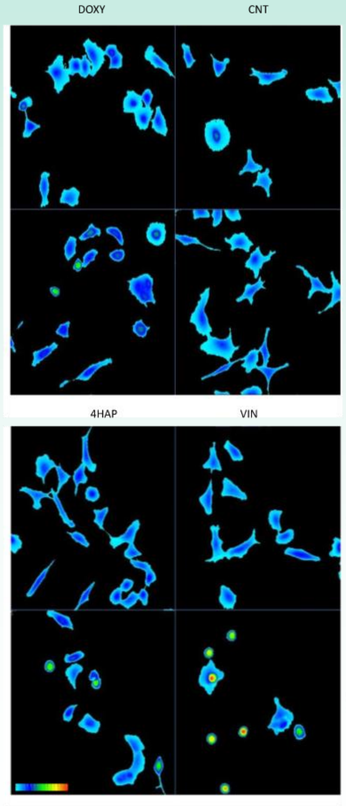
Abstract

Lung cancer cells in vitro, exposed to treatment with putative anti-metastatic agents designated as migrastatics, were examined by time-lapse hiQPI providing measurements of their growth and motility with unprecedented accuracy. © 2021 The Authors

Term MIGRASTATICS

- a specific category of antiinvasive and antimetastatic drugs
- term "migrastatics" is intended for drugs that interfere with all forms of invasion and metastasis of cancer cells and to distinguish this class from conventional cytostatics, which are mainly directed against cell proliferation. [1]

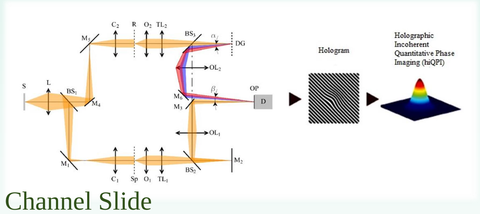


Example processed images taken from 20-hour multifield time-lapse CCHM recordings with the A549 cells. *The potential migrastatics were added before the start. In the control sample, the cells divided, as well as after application of 4HAP and DOXY. In contrast, VIN interfered with cell division. Lapse-interval was 5 minutes. Objective lens was 10× NA 0.3. Field size is 376 µm ×376 µm. Pseudocolor scale represents dry-mass density form 0 (cyan) to 2.1 pg µm^{-1} (red).*

Matherials

- The following three potential migrastatics,
  - vincristine (VIN)
  - doxycycline (DOXY)
  - 4-hydroxyacetophenone (4HAP)
- Concetrations of these three potential migrastatics
  - VIN (100 nM)
  - DOXY (1 mg ml^{-1})
  - 4HAP (4 µM)
- Cell line
  - A549 (the adenocarcinoma human lung alveolar basal epithelial cells)
- Time-lapse
  - 20 hours and 5-min interval
- Microscope
  - Coherence Controlled Holographic Microscope (CCHM)
    - access to Holographic Incoherent Quantitative Phase Imaging (hiQPI) prototype microscope implemented by Tescan Orsay Holding and plan to acquire a new generation hiQPI from Telight Brno.

  - Patented optical set up



- Channel Slide
  - Ibidi µ-Slide VI$^{0.4}$
- Medium
  - standard Eagle MEM medium with 10% fetal bovine serum, 20 µM gentamicin and 200 mM L-glutamine.
  - the medium was enriched with 20 mM TES to maintain pH 7.4
  - Temperature of 37°C was maintained in the environmental enclosure of the CCHM

Results

The CCHM defines the pattern of cell polarity by evaluating the morphological profile of migrating cells along with measuring their distribution in the nucleus and peripheral dry-mass.

- From the hiQPI recordings, we evaluated mean mass doubling time ± S.E.M

Migrastatic	Mass doubling time	ANOVA
CNT	29.2 ± 1.8	
VIN	47.0 ± 3.6	< 0.001
DOXY	35.7 ± 2.5	> 0.05
4HAP	41.9 ± 3.8	> 0.05

- Only VIN reduced growth increasing dry-mass doubling time significantly

Rose plots of cell trajectories from hiQPI recordings. *Individual 150-min tracks were shifted to the common origin. Axis represent distances in µm.* ***A)*** *CNT (n = 3312),* ***B)*** *VIN (n = 5386),* ***C)*** *DOXY (n = 2989) and* ***D)*** *4HAP (n = 4557).*

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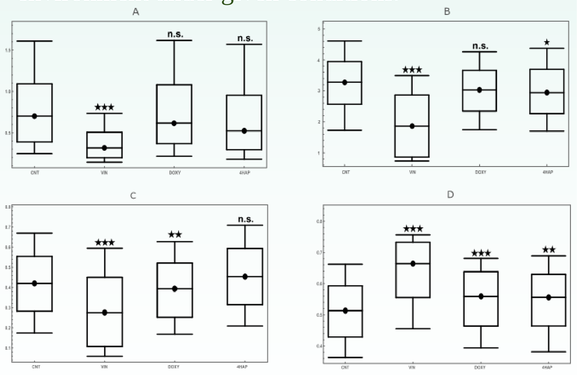

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Conclusions

- The main idea of this project
  - to prove that hiQPI is a reliable and economical approach to in vitro testing of potential migrastatics so that they can be considered for the use in clinics.
- The hiQPI is a realistic candidate for this role
- The most ambitious aspect of the project
  - the highly quantitative analysis of cell motility as related to growth at the individual cell level measuring the intrinsic intra-tumor heterogeneity of cancer cells.
- The critical aspect will then be the discovery of migrastatic influence on various cohorts within the intrinsic heterogeneity of cancer cells.
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Reference

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