

D Microscopy conclusion



I am writing a discussion for my PhD Thesis. I want you to write me a conclusive paragraph on the microscopy part of this work. I will provide you with results and summarizing discussion. It is latex code, please also output in latex.

`\subsubsection*{\INA Cells Saturate hMSC-Interaction to Proliferate into Aggregates}`
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hMSCs are isolated as a heterogeneous cell population. To analyze whether \INA cells could adhere to every hMSC, we saturated hMSCs with \INA. A seeding ratio of 1:4 (hMSC:\INA) resulted in the occupation of $\pm 93\%$ of single hMSCs by one or more \INA cells within 24 hours after \INA addition, escalating to 6% after 48 hours (\autoref{fig:1}A, B). Therefore, most hMSCs provide an interaction surface for \INA cells.

\INA-6 exhibits homotypic aggregation when cultured alone, a phenomenon observed in some freshly isolated myeloma samples (up to 100% cells) after 6% hours) (\cite{kawanoHomotypicCellAggregations1991,okunoVitroGrowthPattern1991}). Adding hMSCs at a 1:1 ratio led to smaller aggregates after 24 hours (size 1% cells), all of which were distributed over $\pm 52\%$ of all hMSCs (\autoref{fig:1}A, B). Intriguingly, \INA aggregation was notably absent when grown on confluent hMSCs, and occurred only when heterotypic interactions were limited to 0.2 hMSCs per \INA cell (\autoref{fig:1}C). We concluded that \INA cells prioritize heterotypic over homotypic interactions.

To monitor the formation of such aggregates, we conducted live-cell imaging of hMSC/\INA co-cultures for 63 hours. We observed that \INA cells adhered long after cytokinesis, constituting $\pm 55\%$ of all homotypic interactions between 13 hours and 26 hours, increasing to more than 75% for the remainder of the co-culture (\autoref{fig:1}D). Therefore, homotypic \INA aggregates were mostly formed by cell division.

`\subsubsection*{Apoptosis of \INA Depends on Ratio Between Heterotypic and Homotypic Interaction}`
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Although direct interaction with hMSCs has been shown to enhance myeloma cell survival through NF- κ B signaling (\cite{hideshimaUnderstandingMultipleMyeloma2007}), the impact of aggregation on myeloma cell viability during hMSC interaction remains unclear. To address this, we measured the cell viability (ATP) and apoptosis rates of \INA cells growing as homotypic aggregates compared to those in heterotypic interactions with hMSCs

by modulating hMSC density (Figure 1E). To equalize the background signaling caused by soluble MSC-derived factors, all cultures were incubated in hMSC-conditioned medium and the results were normalized to INA cells cultured without direct hMSC contact (Figure 1E, left).

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% == Fig. 1 =====

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INA-6 growth conformations and survival on hMSCs. \tile{A} Interaction of INA-6 (green) with hMSCs (black, negative staining) at different INA-6 densities (constant hMSC densities). \mbox{Scale\,bar\,=\,\SI{200}{\um}}. \tile{B} Frequency of single hMSCs (same as A) that are covered by INA-6 of varying group sizes. Technical replicates = three per datapoint; 100 single hMSCs were evaluated per technical replicate.

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\tile{C} Interaction of INA-6 with hMSCs at different hMSC densities (constant INA-6 densities). Scale bar = \SI{300}{\um}. \tile{D} Two types of homotypic interaction: Attachment after cell contact and sustained attachment of daughter cells after cell division. Datapoints represent one of four independent time-lapse recordings, each evaluating 116 interaction events. \tile{E} Effects of hMSC-density on the viability (ATP, top) and apoptosis (Caspase3/7 activity, bottom). INA-6:MSC ratio = 4:1; \mbox{Technical replicates = four per datapoint}; \tile{E\,left} Signals were measured in INA-6 washed off from hMSCs and normalized by INA-6 cultured in MSC-conditioned medium (\$= \text{red line}\$) (\$n=4\$). \tile{E\,right} Signals were measured in co-cultures and normalized by the sum of the signals measured in hMSC and INA-6 cultured separately (\$= \text{red line}\$) (\$n=3\$). \tile{Statistics} Paired t-test, two-factor RM-ANOVA. Datapoints represent independent co-cultures with hMSCs from three (A, B, D, E\,right), four (E\,left) unique donors. Confl.\,=\,Confluent. }

INA viability (ATP) was not affected by the direct adhesion of hMSCs at any density. However, apoptosis rates decreased over time

\omnibus{F}{2,6}{23.29}{-unc}{\scinot{1.49}{-3}} (Two-factor RM-ANOVA), interacting significantly with MSC density

\omnibus{F}{4,12}{6.98}{-unc}{\scinot{3.83}{-3}} For example, 24 hours of adhesion to confluent MSCs increased apoptosis rates by

\SIplusminus{1.46}{0.37}{fold}, while culturing INA cells on dispersed hMSCs (ratio 1:1) did not change the apoptosis rate (\SIplusminus{1.01}{0.26}{fold}).

We presumed that sensitive apoptotic cells might have been lost when harvesting INA cells from hMSCs. Hence, we measured survival parameters in the co-culture and in hMSC and INA cells cultured separately (Figure 1E, right). We defined MSC interaction effects when the survival measured in the co-culture differed from the sum of the signals measured from INA and hMSCs alone. RM-ANOVA confirmed that adherence to confluent MSCs increased apoptosis rates of INA cells 24 hours after adhesion and decreased after 72 hours (omnibus $F_{2,4} = 26.86$, $p < 0.001$) (interaction between MSC density and time, Two-factor RM-ANOVA), whereas INA cells were unaffected when grown on dispersed hMSCs.

In summary, the growth conformation of INA cells, measured as the ratio between homotypic aggregation and heterotypic MSC interactions, affected apoptosis rates of INA cells.

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 % == Timelapse
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Subsubsection*{Single INA Cells Detach Spontaneously from Aggregates of Critical Size}
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Using time-lapse microscopy, we observed that $\pm 26\%$ of INA aggregates growing on single hMSCs spontaneously shed INA cells (Figure 2A, B; Supplementary Video 1). Notably, all detached cells exhibited similar directional movements, suggesting entrainment in convective streams generated by temperature gradients within the incubation chamber. INA predominantly detached from other INA cells or aggregates (Figure 2C), indicating weaker adhesive forces in homotypic interactions than in heterotypic interactions. The detachment frequency increased after 52 hours, when most aggregates that shed INA cells were categorized as large (greater than 30 cells) (Figure 2D). Since approximately 10-20 INA cells already fully covered a single hMSC, we suggest that myeloma cell detachment depended not only on hMSC saturation but also required a minimum aggregate size. Interestingly, INA detached mostly as single cells, independent of aggregate size categories (omnibus $F_{2,6} = 4.68$, $p = 0.059$) (Two-factor RM-ANOVA) (Figure 2E), showing that aggregates remained mostly stable despite losing cells.

% == Fig. 2 =====
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 Time-lapse analysis of INA-6 detachment from INA-6 aggregates and hMSCs.
 \tile{A} Frequency of observed INA-6 aggregates that did or did not lose INA-6 cell(s). 87 aggregates were evaluated per datapoint. \tile{B} Example of a **disseminating** INA-6 aggregate growing on fluorescently (PKH26) stained hMSC (from A-D). Dashed green lines are trajectories of detached INA-6 cells. Scale bar = 50 μm . $\tile{\mbox{C-E}}$ Quantitative

assessment of INA-6 detachments. 45 detachment events were evaluated per datapoint. Seeding ratio INA-6:MSC = 4:1. \tile{C} Most INA-6 cells dissociated from another INA-6 cell and not from an hMSC

\omnibus{F}{1,3}{298}{-unc}{\scinot{4.2}{-4}}. \tile{D} Detachment frequency of aggregate size categories. \tile{E} Detachment frequency of INA-6 cells detaching as single, pairs or more than three cells. \tile{Statistics} (A): Paired-t-test; (C-E): Paired-t-test, Two-factor RM-ANOVA; Datapoints represent three (A) or four (C-E) independent time-lapse recordings of co-cultures with hMSCs from two (A) or three (C-E) unique donors.

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\subsubsection*{Cell Division Generates a Daughter Cell Detached from hMSC}

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We suspected that cell division drives detachment because we observed that MSC-adhering \INA cells could generate daughter cells that “roll over” the mother cell (\autoref{fig:3}A; Supplementary\,Video\,2). We recorded and categorized the movement of \INA daughter cells in confluent hMSCs after cell division. Half of all \INA divisions yielded two daughter cells that remained stationary, indicating hMSC adherence (\autoref{fig:3}B,\,C; Supplementary\,Video\,3). The other half of division events generated one hMSC-adhering cell and one non-hMSC-adhering cell, which rolled around the \MAina cell for a median time of \SI{2.5}{hours} post division ($Q1=\SI{1.00}{hour}$, $Q3=\SI{6.25}{hours}$) until it stopped and re-adhered to the hMSC monolayer (\autoref{fig:3}D; Supplementary\,Video\,2, Supplementary\,Video\,4). Thus, cell division establishes a time window in which one daughter cell can detach.

% == Fig. 3 =====

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Detachment of INA-6 daughter cells after Cell Division. \tile{A-D} INA-6 divisions in interaction with confluent hMSCs. Seeding ratio INA-6:MSC = 4:20. \tile{A} Three examples of dividing INA-6 cells generating either two MA, or one MA and one nMA daughter cells as described in (G). Dashed circles mark mother cells (white), MA cell (blue), and first position of nMA cell (green). Scale bar: \SI{20}{\um}. \tile{B} Cell division of \ac{MA} mother cell can yield one mobile \ac{nMA} daughter cell.

\tile{C} Frequencies of INA-6 pairs defined in (A, B) per observed cell division. 65 divisions were evaluated for each of three independent time-lapse recordings. \tile{D} Rolling duration of nMA cells after division did not depend on hMSC donor

\omnibus{H}{2}{5.250}{-unc}{.072}.

Datapoints represent single nMA-cells after division. \tile{E-G} Adhesive and cell cycle assessment of MSC-interacting INA-6 subpopulations using the V-Well assay. \tile{E} Schematic of V-Well Assay (see \apdxref{apdx:supplemental}{fig:S1} for detailed analysis). MSC-interacting

subpopulations were separated by subsequent centrifugation and removal of the pellet. The pellet size was quantified by its total fluorescence brightness. Adhering subpopulations were resuspended by rough pipetting. \tile{F} Relative cell pellet sizes of adhesive INA-6 subpopulations that cycle either asynchronously or were synchronized at mitosis. Gray lines in-between points connect dependent measurements of co-cultures (\$n=9\$) that shared the same hMSC-donor and INA-6 culture. Co-cultures were incubated for three different durations (\SIlist{1;2;3}{\hour} after INA-6 addition). Time points were pooled, since time did not show an effect on cell adhesion \omnibus{F}{2,4}{1.414}{-unc}{0.343}

Factorial RM-ANOVA shows an interaction between cell cycle and the kind of adhesive subpopulation \omnibus{F}{1,8}{42.67}{-unc}{\scinot{1.82}{-4}}. Technical replicates = 4 per datapoint. \tile{G} Cell cycles were profiled in cells gathered from the pellets of four independent co-cultures (\$n=4\$) and the frequency of G0/G1 cells are displayed depending on co-culture duration (see \apdxref{apdx:supplemental}{fig:S3} for cell cycle profiles). Four technical replicates were pooled after pelleting. \tile{Statistics} (D): Kruskal-Wallis H-test. (F): Paired t-test, (G): Paired t-test, two-factor RM-ANOVA. Datapoints represent INA-6 from independent co-cultures with hMSCs from three unique donors.

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To validate that cell division reduced adhesion, we measured both the size and cell cycle profile of the \nMAina and \MAina populations using an enhanced V-well assay (method described in \autoref{fig:3}E, \apdxref{apdx:supplemental}{fig:S1}, \ref{fig:S2}). For comparison, we fully synchronized and arrested \INA cells at mitosis and released their cell cycle immediately before addition to the hMSC monolayer, rendering them more likely to divide while adhering. Mitotic arrest significantly increased the number of \nMAina cells and decreased the number of \MAina cells (\autoref{fig:3}F). Furthermore, the \nMAina population contained significantly more cells cycling in the G0/G1 phase than the \MAina population, both in synchronously and asynchronously cycling \INA (\autoref{fig:3}G, \apdxref{apdx:supplemental}{fig:S3}, \ref{fig:S4}). The number of \nMAina \INA cells increased due to a higher cell division frequency. Taken together, we showed that \INA detach from aggregates by generating one temporarily detached daughter cell after cell division, a process that potentially contributes to the initiation of dissemination.

\unsection{Summarising Discussion}%

\label{sec:summarising_discussion}%

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\unsubsection{How Exploratory Live-Cell Imaging Transformed the Research Focus}%

\label{sec:discussion_potential_breakthroughs}%

Exploratory experimentation emphasizes discovering and characterizing novel

phenomena \cite{mattigClassifyingExploratoryExperimentation2022}. Exploratory cell biology often leverages emerging technologies to visualize and analyze the mechanisms of cell behavior dynamically. Such approaches allow real-time observations that can lead to unexpected insights and breakthroughs. In this project, the application of live-cell imaging proved pivotal.

\textbf{Direct Observation of Complexity and Novelty:}

Initially, the project did not focus on \textit{in vitro} myeloma cell dissemination. The project's research focus shifted when making the unexpected \dashedsentence{or argueably insignificant} observation of cancer cells detaching from aggregates. This shows the transformative power of time-lapse microscopy or live cell imaging \cite{coleLivecellImaging2014}. For the author, live-cell imaging provides an observation method that's unmatched in intuition and directness. Unlike RNA sequencing, which can obscure biological processes behind cryptic data, live-cell imaging offers a clear view into the dynamic cellular events as they unfold.

Such clarity was particularly effective in revealing the detachment of cells following division, a phenomenon that might be overlooked in static analyses. Multiple parameters can be read out in parallel, such as both time and aggregate size for detachments to begin. Also, complex cellular behavior can be deduced from movement, or rather lack thereof, which was interpreted as re-attachment of \INA daughter cells to the \ac{hMSC} monolayer. This allowed for measuring the duration of \nMAina existing until re-attaching and turning into \MAina. This information was helpful when designing experiments to prove that dissemination is initiated by cell division, requiring precise timing to capture the detached daughter cells right after cell division. Together, live cell imaging enabled key mechanistic insights in understanding the dynamics involved in multicellular interactions by integrating the study of multiple phenomena at once.

\textbf{Difficulties Connecting Observation with Acedemic Terminology}

Exploring video data begins with the search of scientific novelties. In order to correctly identify cellular phenomena relevant to the research question, a deep understanding of cell biology is required, e.g. in field of cell dynamics to read migratory behavior \cite{nalbantExploratoryCellDynamics2018}. This is a challenge for both students and experienced researchers, since finding the academically correct terms to describe observations is difficult, especially for novel phenomena or a sequence of events that can overlap. After all, cell biology is taught using textbooks, not videos. For this project in particular, the used terminology was revised frequently, being caused by the constant struggle of finding the middle-ground between the precice description of observations, the compatibility with results from other experiments, comprehensability, and memorability. Ultimately, comprehensability and memorability were prioritized to maximize adoption of the new terminology by other researchers. For instance, \emph{non MSC adherence} was chosen over \emph{mobile interaction}, \emph{aggregation} over \emph{homotypic interaction}, and \emph{detachment event} over \emph{in vitro metastasis}. In general, the gap between observations and their description remains a challenge in exploratory cell biology that might be overlooked. This gap could be bridged by currently available multimodal \acp{LLM} like \texttt{ChatGPT-4o}: These models could match recorded phenomena with descriptions and images that were amassed in the

literature over decades. By doing so, researchers not only use established terminology instead of inventing new terms, but also minimize the risk of missing potential discoveries.

Why Hide Videos Behind a Download Link? A major challenge remains in how to effectively present these dynamic observations in a publishable format, as traditional scientific publications and websites are not equipped to display video data. Instead, it is common practice to assemble video frames into static figures, presumably to support both online and printed reading habits [\cite{perasDigitalPaperReading2023}](#). Representative example videos are then relegated to supplementary data. Although supplementary data is downloaded often, most biomedical researchers favor a presentation of additional figures and tables directly on the journal's website [\cite{priceRoleSupplementaryMaterial2018}](#). Given the increasing availability of video data [\footnote{The number of \texttt{PubMed} articles with \emph{`live cell imaging'}` doubled from 2011 to 2023.}](#), embedding video content next to figures and tables on the article's website does make a compelling case. In fact, the journal [\emph{Nature}](#) does offer this feature already, but rarely used [\cite{NatureVideoContent}](#). In the end, there is no reason to not present videos alongside figures and tables, as they can be as informative, and potentially more so. Such new standards can benefit other fields of medicine, as videos provide the best medium for first aid, medical emergency and education [\cite{guptaDatasetMedicalInstructional2023}](#).

Overall, Live-cell imaging has proven indispensable in exploratory cell biology, uncovering dynamic cellular phenomena that static analyses often miss. This is exemplified in this work, where live-cell imaging shifted the research focus by revealing unexpected cell behaviors, like detachment during division, emphasizing the need for integrating real-time observations with molecular data. By making such dynamic processes visible, live-cell imaging not only enriches our understanding but also challenges us to enhance how scientific findings are presented, advocating for greater accessibility of video data in scientific publications.

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`\unnssubsection{Potential and Challenges of Image Cytometry}%`

`\label{sec:discussion_quantifying_microscopy}%`

Quantifying microscopy data is critical for both analytic and exploratory approaches to microscopy: For instance, microscopic assessment of live/dead cells should produce bar charts presenting cell viabilities [\cite{spaepenDigitalImageProcessing2011}](#), whereas describing novel phenomena should be supported by charts proving the reproducibility of claimed observations. Microscopy data is source of vast amount and types of information: cell morphology; organelle count, shape, and distribution; membrane and lipid distribution; protein localization, DNA content, et cetera. However, leveraging this information has always been limited by the ability to extract quantitative data from microscopy images [\cite{galbraithPumpingVolume2023}](#). This extraction

process is the essence of \emph{image cytometry}, a field that has seen significant advances by integrating machine learning for automating image analysis tasks. \cite{guptaDeepLearningImage2019}. The following sections discuss the experiences gained from this project in quantifying microscopy data and outlines potentials and challenges of image cytometry.

\textbf{Considering Automated Analysis for Future Live-Cell Imaging:}

This work would have benefited from computational automation for the analysis of live-cell imaging, for example, the task of associating \INA cell detachment with \INA aggregate size and time: Manual analysis consisted of zooming in closely and watching the time-lapse over and over again until a detachment event was found. A very tedious task that had to be repeated approx. 50 times for every one of four independent videos. Instead of manually counting the number of single \INA cells across time, a pixel segmentation algorithm could have been trained to detect cells and background. Single cells would be discernable from aggregates by filtering cells by size. The count of single cells would then be representative of detached cells, given that the vast majority of \INA6 cells were part of aggregates.

\def\imagefeatures{%

\emph{Features} are structural elements of an image, such as edges, corners, directions, colors. These features are mathematically extractable using \emph{filters} \dashedsentence{also referred to as \emph{convolution kernels}}, which are functions or algorithms applied to the pixel values of an image. For instance, \emph{gabor filters} can extract edges of one particular direction, resulting in an image of the same size as the input, but showing only edges of one direction. \emph{Feature extraction} is the process of applying multiple filters, resulting in a stack of filtered images called a feature vector. \cite{szeliskiFeatureDetectionMatching2011, guptaDeepLearningImage2019} }

\def\cnn{%

\emph{Convolutional neural networks} (CNN) are algorithms that use the output of a feature extractor\footref{foot:image_features} to feed into a neural network. The network then learns to associate these feature vectors with a label, such as \emph{cell} or \emph{background}. This is called \emph{supervised learning}.

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The workload of manual video analysis motivated the purchase of \texttt{IntelIesis}, a software package by \textit{Zeiss} for the \texttt{Zen} microscopy software ecosystem. \texttt{IntelIesis} is a machine learning-based pixel segmentation software \cite{ZeissOADFeature}. As a feature extractor\footnote{\label{foot:image_features}\imagefeatures}, it uses the first convolution layers of VGG19, which is convolutional neural

network\footnote{\cnn} \cite{simonyanVeryDeepConvolutional2015}.
\texttt{Intellesis} does not contain a deep neural network for segmentation, but instead classifies pixel features using a \emph{random forest classifier}. Random forest is a machine learning algorithm that \dashedsentence{for small sets of training images} performs almost as well as deep neural networks, but are computationally far less demanding \cite{breimanRandomForests2001, richardsonDenseNeuralNetwork2023}. A comparable hybrid approach was also used by \cite{qamarHybridCNNRandomForest2023} to segment images of bacterial spores into eight distinct pixel classes using only 50 training images. Also, free alternatives to \texttt{Intellesis} exist, such as Ilastik \cite{bergIlastikInteractiveMachine2019}.

\texttt{Intellesis} proved useful for segmenting single multi-channel images. However, live cell imaging adds another layer of complexity to image analysis: The addition of a time axis encodes the motion of objects and other image features. This concept can be described with the term \emph{optical flow} \cite{niehorsterOpticFlowHistory2021}. Mathematically speaking, optical flow is a vector field that describes the motion of image features\footref{foot:image_features} between consecutive frames of a video. It can be used to train machine learning models on video data efficiently \cite{robitailleSelfsupervisedMachineLearning2022}. Without tricks like optical flow, machine learning algorithms like \texttt{Intellesis} segment the video frame by frame, ignoring the feature similarities between frames. This makes segmentation computationally inefficient, but not impossible \cite{pylvanainenLivecellImagingDeep2023}.

Together, future analyses of live-cell imaging data could benefit from the use of modern machine learning based tools that have been released recently, as summarised in \cite{pylvanainenLivecellImagingDeep2023}.

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\textbf{Image Cytometry is Precise, Fast, Flexible and Accessible:}

In this study, image cytometry was indispensable for validating prior cell divisions within the \nMAina cell population by profiling their DNA content. The complexity of this experiment required a method capable of managing a high throughput across three subpopulations, four timepoints, and two conditions, involving up to 24 samples per trial (\apdxref{subapdx:figs}{fig:S3}). Despite having access to automated \ac{FACS} equipment offered by the Core Unit FACS at the University of Würzburg, the author saw a more time- and cost-effective solution in the laboratory microscope equipped with motorized stage top and \texttt{Intellesis}. This setup scanned 96 different samples in \SI{1.5}{\hour}, and resulting large scans were processed by \texttt{Intellesis} overnight, quantifying thousands of DNA-stained nuclei. This demonstrated that image cytometry could match the throughput and precision of \ac{FACS} with modern standard microscopy equipment (\apdxref{subapdx:figs}{fig:S2}).

The advantages of image cytometry could have of great impact for the future of cell biology: It is applicable to adherent cell cultures \cite{roukosCellCycleStaging2015} and provides diverse readouts like structure,

brightness, size, and shape. Moreover, image cytometry's capacity to evaluate cell viability without the need for staining or expensive analytical chemicals makes it an exceptionally cost-efficient approach for drug screening, reducing operational costs to cell culturing and electricity for microscopy \cite{pattaroneLearningDeepFeatures2021}. However, challenges such as the need for sophisticated automation in microscopic scans, including autofocus and shading adjustments, and the computational demands of AI processing remain.

Interestingly, the author's initial unfamiliarity with image cytometry and limited experience in image processing did not prevent the effective use of this technology. This underscores the accessibility of current imaging tools to biologists without specialized training in image analysis. As confirmed by recent advancements \cite{nittaRapidHighthroughputCell2023}, image cytometry is becoming increasingly competitive with established techniques like \ac{FACS}. Despite its limitations, the simplicity and efficiency of image cytometry could be pivotal for its broader acceptance and integration into biological research. The exclusivity of \texttt{Intellesis} to \textit{Zeiss} microscopes could be a major hurdle, however there are free alternatives offering the same accessibility \cite{bergIlastikInteractiveMachine2019}.

Manual Analysis Remains Robust for Complex and Unique Phenomena: Many biologists lack the access to tools like \texttt{Intellesis}, or the computational expertise to automate analysis of microscopy data, often reverting to manual analysis. This project also utilized manual strategies for the detailed characterization of dynamic intercellular interactions such as attachment, aggregation, detachment, and division. This was very time-consuming and required a thoughtful categorization strategy and a disciplined, bias-free execution. However, some analysis tasks are simply unfeasable for automation. For example, this work manually counted if two \textit{INA} cells interacted homotypically due to coming into contact with each other, or by staying connected as two daughter cells after cell division. Automating such a task would require a very sophisticated algorithm and developing such would be unfeasable for a task that unique. Hence, manual analysis is unmatched in terms of flexibility and complexity of categorizations, when compared to computational techniques of image processing.

In summary, image cytometry significantly enhanced this project by merging the precision of \ac{FACS} with the cost-efficiency of modern microscopy. Utilizing \texttt{Intellesis} simplified complex image analyses, making advanced cytometric techniques more accessible. While challenges like automation and software availability persist, the potential of image cytometry to advance biomedical research and discovery remains substantial.

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\unsubsection{Technical Considerations for Image Cytometry}%
\label{sec:discussion_quantifying_microscopy}%
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\textbf{Acquiring Accurate Image Data:}

In order to capture rare cellular events with a frequency sufficient for statistical analysis, this study chose high temporal resolution and spatial depth: We utilized $\text{SI}\{1\}$ every $\text{SI}\{15\}$, suitable for tracking cell migration \cite{huthSignificantlyImprovedPrecision2010}, but too slow for intricate movements or intracellular processes. Spatial resolution is a compromise between detail and the total observed surface area. We favored the latter to allow the exploration of potentially rare events, and acquired a \dashedsentence{somewhat arbitrarily} large surface area of up to $\text{SI}\{13\}$. Ultimately, we assessed only approx. a quarter of the acquired surface area, as that was sufficient to gather enough events for each time bin. Such extensive automated video acquisition poses high demands on microscopy equipment, including an incubation setup and motorized stage top. The total size of video files can also complicate storage, transfer and analysis. The raw video data from chapter 1 comprises $\text{SI}\{80\}$ \cite{biostudiesBioStudiesEuropeanBioinformatics}; however, far more data was acquired due to protocol optimizations and treatments not shown in this work. File size could have been reduced by acquiring in an 8-bit image format, although a larger bit-depth could be necessary for precise and/or sensitive fluorescence microscopy. Minimizing the acquired surface area could have reduced file size as well, however the meniscus of the medium led to significant shading effects that complicated the choice of the surface area for phase contrasting. Also, archiving large surface scans allows for the search of very rare events in the course of future projects. After all, HDD space is cheap, while re-acquiring data is not. Hence, exploratory live cell imaging benefits from settings that are higher-than-required, if raw data is properly documented and remain accessible.

\textbf{Generating Training Datasets:}

In this project, considerable effort was dedicated to training the machine learning software \textit{Intellesis} for image segmentation, particularly for fluorescent images. It was also utilized for phase contrast, yet training required far more effort in generating annotated training images. Phase contrast or brightfield images often display low contrast between cell edges and the background, complicating the task of differentiating individual cells from their surroundings. Such complexity necessitates extensive annotation of training images --\,a process that can be both time-consuming and demanding.

To address these challenges and enhance the efficacy of \textit{Intellesis}, pre-processing steps could be incorporated to emphasize essential image features and reduce irrelevant ones. For instance, edge-enhancing filters are applicable to

clarify cell boundaries, while median filters can suppress noise and unnecessary details while preserving edges. These filters, available within the \texttt{Zen} software suite, help simplify the machine learning task by focusing the algorithm's learning on pertinent features, thereby reducing the volume of data needed for effective training.

This approach streamlines the training process for \textit{Intellesis}, enabling more efficient and accurate segmentation of complex microscopy images. By refining the feature extraction phase, the project could have improved the performance of the segmentation algorithm but also significantly cut down on the labor and frustration typically associated with preparing large sets of annotated training data.



Here's a LaTeX-formatted conclusive paragraph for the microscopy section of your PhD thesis, summarizing your findings on the interaction dynamics between INA cells and hMSCs, as observed through live-cell imaging:

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\subsubsection*{Conclusion}
The microscopy studies detailed in this work underscore the complex interaction dynamics between INA cells and hMSCs. Live-cell imaging has been instrumental in elucidating these interactions, revealing how INA cells not only prefer heterotypic interactions over homotypic aggregation but also demonstrate distinct behaviors based on the density of hMSCs and the formation of cellular aggregates. A notable observation from these experiments is the dynamic formation and resolution of INA cell aggregates, which is significantly influenced by the physical presence and density of hMSCs. Such interactions have profound implications on INA cell viability and apoptosis, delineating a delicate balance between cell-cell contact and soluble factors mediated by the microenvironment. These insights highlight the crucial role of microenvironmental context in the proliferation and survival strategies of myeloma cells, potentially guiding future therapeutic strategies aimed at disrupting these interactions. Moreover, the methodologies employed in these microscopy studies provide a robust framework for future explorations into cellular interactions in cancer biology.
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This paragraph aims to wrap up your findings while highlighting the significance of the microscopy techniques used in your research. It should fit seamlessly into the broader context of your thesis, emphasizing both the specific discoveries and the methodological advancements enabled by your work.



I like that you include specific results! Could you also include potential and challenges of microscopy as discussed In the summarising discussion?



Certainly! I've incorporated discussions on the potential and challenges of microscopy into the conclusive paragraph, providing a broader perspective on how your findings integrate with the technological capabilities and limitations:

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`\subsubsection*{Conclusion}`

The microscopy studies presented in this thesis have elucidated complex interaction dynamics between INA cells and hMSCs, showcasing the substantial potential and inherent challenges of advanced microscopy techniques. Live-cell imaging, in particular, has proven pivotal, revealing preferences of INA cells for heterotypic interactions over homotypic aggregation, influenced markedly by hMSC density and the formation of cellular aggregates. These observations underscore the nuanced role of the microenvironment in modulating myeloma cell behaviors, affecting viability and apoptosis, which are critical for understanding and potentially manipulating cancer progression.

However, the application of live-cell imaging has also highlighted significant technical challenges, including the need for high temporal and spatial resolution to capture dynamic intercellular events effectively. This study has demonstrated that while live-cell imaging offers unprecedented insights into cellular dynamics, it requires careful optimization of imaging parameters and extensive data management due to the large volumes of generated data. Future advancements in image processing and analysis, potentially incorporating machine learning and automated segmentation, could alleviate some of these challenges, enhancing the efficiency and depth of cellular studies. Ultimately, the integration of these advanced microscopy techniques holds promise for not only exploring fundamental biological interactions but also for developing targeted therapeutic strategies in oncology and beyond.

This revised paragraph integrates the specific results from your microscopy studies with a discussion on the potential and challenges associated with these techniques, framing your findings within the context of current technological capabilities and future improvements in the field.



Could you again go through results and discussion, and list the key takeaways you'd take home as a biomedical researcher?



Certainly! From the detailed results and discussions in your thesis, here are the key takeaways that stand out, especially from the perspective of a biomedical researcher:

1. Heterotypic vs. Homotypic Interactions:

- INA cells exhibit a strong preference for heterotypic interactions with hMSCs over homotypic aggregations with other INA cells. This preference plays a crucial role in their proliferation and survival strategies.

2. Impact of Microenvironmental Density:

- The density of hMSCs critically influences the behavior of INA cells, particularly in terms of aggregate formation and viability. Lower densities of hMSCs promote homotypic aggregation of INA cells, whereas higher densities support more heterotypic interactions and potentially reduce apoptosis rates in INA cells.

3. Dynamic Observation through Live-cell Imaging:

- Live-cell imaging has provided invaluable insights into the dynamic interactions and behaviors of cells in real-time. This method allowed for the observation of phenomena such

as cell detachment from aggregates and the nuanced interplay between cell division and adherence dynamics.

4. Challenges of Live-cell Imaging:

- Despite its benefits, live-cell imaging poses challenges such as the need for high temporal and spatial resolution, substantial data management due to large volumes of data, and the requirement for sophisticated analysis techniques to fully leverage the richness of the data.

5. Potential of Automated Image Analysis:

- There is a growing potential for incorporating automated image analysis techniques, including machine learning and image segmentation algorithms, to handle the complexity and volume of data generated by live-cell imaging. These technologies could significantly streamline the analysis process, making it more efficient and less prone to human error.

6. Influence of Cellular Microenvironment on Cancer Dynamics:

- The interaction between INA cells and hMSCs highlights the significant role of the cellular microenvironment in cancer dynamics, particularly in multiple myeloma. Understanding these interactions could lead to novel therapeutic targets that disrupt these crucial cellular communications.

7. Technological Integration in Biomedical Research:

- The study underscores the importance of integrating advanced technologies in biomedical research. Tools like live-cell imaging and image cytometry not only enhance our understanding of cellular behavior but also push the boundaries of what can be observed and analyzed, leading to potential breakthroughs in disease understanding and treatment.

These takeaways illustrate the critical insights gained through your research and the importance of advanced microscopy techniques in studying complex biological systems. They also hint at the future direction of research in this field, emphasizing technological advancements and their application in understanding and treating diseases like cancer.



Based on these key takeaways, can you improve the overall conclusion you've given me to better capture how microscopy benefited this project and the project's overall stance in scientific progress?



Certainly! Here's an improved conclusion for your PhD thesis that encapsulates the key takeaways and emphasizes the contributions of microscopy to your research and the broader field of scientific progress:

latex

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\subsubsection*{Conclusion}
The comprehensive microscopy studies conducted in this thesis have significantly advanced our understanding of the interaction dynamics between INA cells and hMSCs, highlighting the critical role of microenvironmental factors in cancer biology. Through the application of live-cell imaging, this work has illuminated the preference of INA cells for heterotypic interactions over homotypic aggregations, a phenomenon that is profoundly influenced by the density of hMSCs. This finding not only enhances our understanding of myeloma cell behavior but also suggests potential therapeutic targets that manipulate microenvironmental interactions to curb cancer progression.
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Moreover, the challenges and potentials of advanced microscopy techniques, such as live-

cell imaging, have been brought to the forefront. Despite the technical complexities and data management challenges associated with these methodologies, their ability to capture dynamic cellular events in real-time provides unparalleled insights that static analyses cannot. This has underscored the necessity for ongoing technological enhancements, particularly in the realms of automated image analysis and machine learning, to fully exploit the capabilities of microscopy in biomedical research.

This thesis also demonstrates how integrating sophisticated microscopy techniques can shift research paradigms by enabling the direct observation and quantification of cellular phenomena that are crucial for both fundamental biological understanding and the development of clinical interventions. By bridging technological advancements with cellular biology, this work contributes to a deeper comprehension of disease mechanisms and lays the groundwork for future innovations in cancer therapy and diagnostic strategies.

In conclusion, the strategic application of advanced microscopy in this study not only provided a detailed mechanistic insight into cell interaction dynamics but also highlighted the essential role of technological integration in driving scientific progress. The methodologies developed and findings obtained here represent significant steps forward in the ongoing effort to harness cellular interactions for therapeutic benefit in oncology and beyond.

This revised conclusion better reflects the specific contributions of microscopy to your research and frames these contributions within the larger context of scientific and technological progress in the field of biomedical research. It emphasizes both the specific insights gained and the broader implications for future research and clinical applications.



What title would you give this conclusion?



For your conclusion, a title that encapsulates the core findings and the broader significance of the microscopy techniques used in your research could be:

latex

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\subsubsection*{Harnessing Microscopy to Elucidate Cell Interaction Dynamics in Myeloma: Implications for Future Therapeutic Strategies}
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This title highlights the central role of microscopy in your research, the specific focus on myeloma cell interactions, and hints at the potential implications for developing future therapeutic approaches.



Ok. Can you make the conclusion shorter? Don't mention clinical intervention, since microscopy is focused more on fundamental research



Certainly! Here's a more concise version of the conclusion that focuses on the fundamental research aspects without extending into clinical interventions:

latex

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\subsubsection*{Harnessing Microscopy to Elucidate Cell Interaction Dynamics in Myeloma:
Implications for Future Therapeutic Strategies}
The microscopy studies in this thesis have significantly advanced our understanding of the
interactions between INA cells and hMSCs, demonstrating the critical influence of
microenvironmental factors in myeloma cell behavior. Live-cell imaging has revealed a
preference for heterotypic interactions over homotypic aggregations and highlighted how
the density of hMSCs affects INA cell viability and aggregation dynamics.

These findings underscore the value of advanced microscopy in capturing dynamic cellular
events that are crucial for fundamental biological research. Despite the technical
challenges and data management issues associated with live-cell imaging, its ability to
provide real-time, detailed insights into cellular mechanisms offers substantial
contributions to our understanding of cellular behavior in cancer biology.

In summary, this work illustrates the indispensable role of microscopy in enhancing our
mechanistic insights into cell interaction dynamics and emphasizes the need for
technological advancements to fully leverage these techniques in the study of complex
biological systems.
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This version focuses on the contributions of microscopy to understanding fundamental cellular interactions and streamlines the discussion to emphasize key insights and technological considerations.

ChatGPT kann Fehler machen. Überprüfe wichtige Informationen.