Cytometry PART A
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Tycho Brahe's Way to Precision

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TYCHO Brahe (1546–1601) was the inventor of precise and reproducible science. My transformation of the novel title by early 20th century writer Max Brodt emphasizes his major impact on performing science the way we do or at least should do today. He for sure was the major astronomer of his time, receiving the island Ven from the Danish king to build there his high end stellar observation instruments (Fig. 1). With substantial financial support from the king he built up stellar observation stations that were the CERN (Conseil Européen pour la Recherche Nucléaire, The European Organization for Nuclear Research) of the 16th century. Although he did his observations by eye (telescopes were not yet invented), the precision of the measurements was so spectacular that they were only improved generations later (Fig. 2). His methods to achieve this precision sound now familiar to us because in experimental science we apply comparable approaches: 1) repeated measures; 2) independent techniques and instruments to test the findings and hypothesis; and 3) precise documentation enabling following generations to use his data for further analysis (1).

In Cytometry Part A we have tried to follow his advice regarding experiment documentation and data availability by introducing the MIFlowCyt annotation for flow cytometry data in 2008 (2) and open access to source data by the FlowRepository in 2010 (3). As then several other journals started to accommodate these formats but so far only Cytometry Part A achieved that; well over 95% of the original research publications with flow cytometry data are compliant and many also provide original source data. Therefore, we decided to go to the next level and make MIFlowCyt mandatory for all applicable manuscripts and request that original list-mode are made publicly available. These changes are now effected and the guidelines for authors have been modified accordingly (4). Importantly, in case of clinical data there are tools available to de-identify the data files so that critical personal data are not disclosed. Looking at possible next development, the Google Dataset Search or comparable initiatives may become useful tools to find open access source data outside of the repository.

The next step for transparency of data and experiment details is the release of a MIFlowCyt equivalent for image cytometry data. This is however no little feat due to the wide range of approaches in image cytometry to measure and analyze samples with a variety of data output formats. Concurrently, this October issue has several research manuscripts using image cytometry for quantitative analyses of cells and tissues which also illustrates the diversity of the field of image cytometry.

The article by Chiang et al. (this issue, page 1004) describes an automated image analysis approach for quantifying vacuoles in bright field microscopy images as an indicator of cell death. The approach detects and counts the number of vacuoles and vacuolated cells with an estimated error of 3.33% absolute error between automated vacuolated cell detection and manual detection, and includes a freely accessible GUI to enable others to easily adopt this technique. This capability for inferring viability and cell death from bright field images could be of particular importance in rapid screening for new chemotherapeutic compounds.

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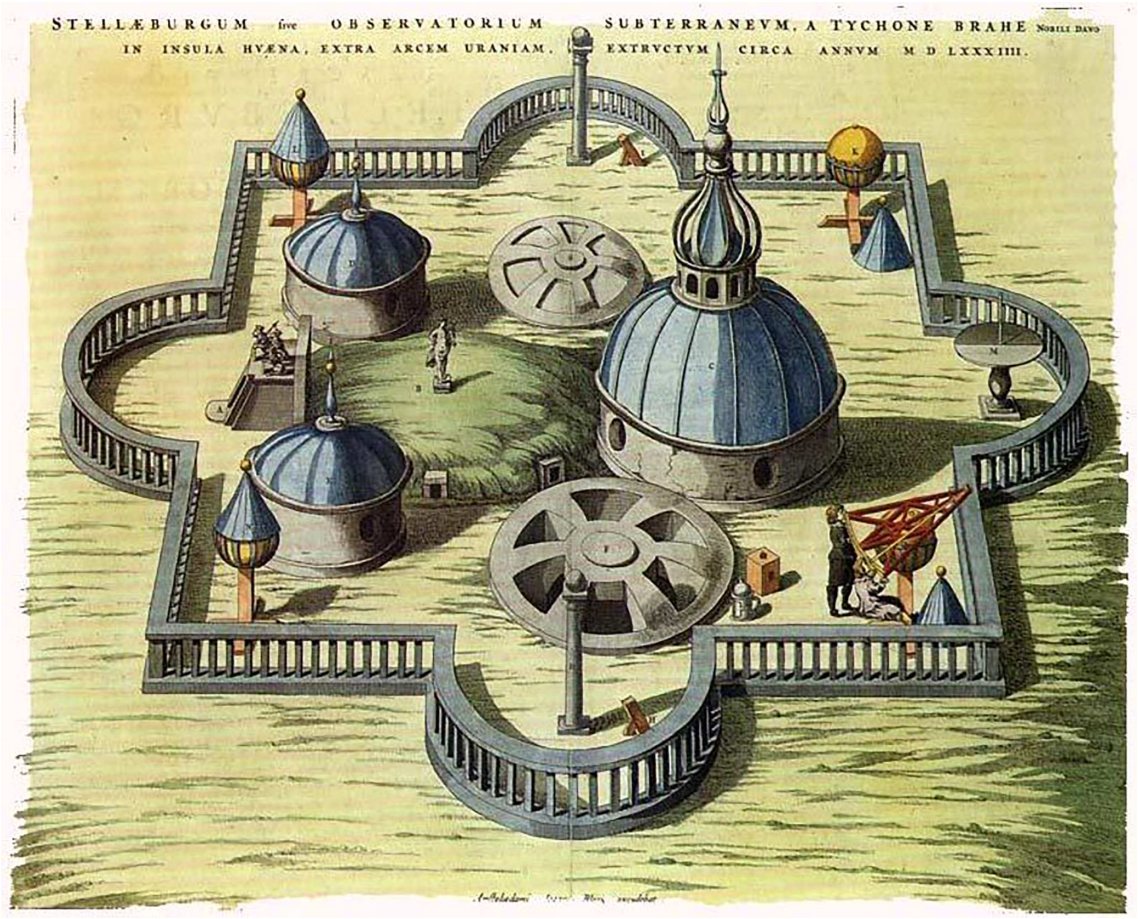

Figure 1. Tycho Brahe's observatory on Ven: Johan Blaeu, Atlas Major, Amsterdam, 1662-DET KONGELIGE BIBLIOTEK. Tycho Brahe's Stjerneborg from Johan Blaeu's Atlas Major, Amsterdam 1662, vol. 1. This image is a drawing by Willem Blaeu of the Stjerneborg observatory circa 1595, it is taken from an Atlas by his son, Johan Blaeu, published in 1662 in Amsterdam (from Wikipedia. This work is in the public domain in its country of origin and other countries and areas where the copyright term is the author's life plus 100 years or less).

Koyuncu and colleagues (this issue, page 1019) also describe a new quantitative image analysis approach—in this case for segmentation of cell nuclei that is especially well suited for overlapping nuclei. The approach defines nuclear edge objects using Sobel filters, then breaks the image into small homogeneous regions, and then recombines those regions into nuclear regions using a scoring system. The results indicate improved performance over prior nuclear segmentation approaches for images with somewhat-to-moderately overlapping nuclei. This approach may improve the accuracy of image cytometry measurements for the range of cell culture lines and conditions that do not result in uniform monolayer growth.

Spectral imaging approaches have been shown to allow separation of competing and overlapping signals. The article by Annamdevula and colleagues (this issue, page 1029) applies spectral imaging for removal of competing background and autofluorescence signals in Förster resonance energy transfer (FRET) image data, allowing improved accuracy for cell segmentation and FRET calculations. The authors also extend the approach into three spatial dimensions (x,y,z) to detect localized spatial gradients in second messenger cAMP signals, where these spatial gradients are believed to play a role in signaling specificity and downstream cell physiological outcome.

The article by Tollemar and colleagues (this issue, page 1051) demonstrates that CellProfiler software (5) can be used for automated analysis of chromogenically stained immunohistochemical (IHC) samples. The approach allows repeatable, unbiased, rapid screening of clinical IHC image data, and provides a high correlation with manual counting for quantification of CD4 stained cells in oral mucosal biopsies. In addition, CellProfiler software allows calculation of additional cell-dependent geometric parameters. While CellProfiler has historically been used for analysis of fluorescence image data, this approach demonstrates that it may also be highly applicable to analysis chromogenic image data.

Zigon and colleagues (this issue, page 1060) detail a method for verifying the results of single cell sorting using an image cytometer with capabilities for automated analysis of

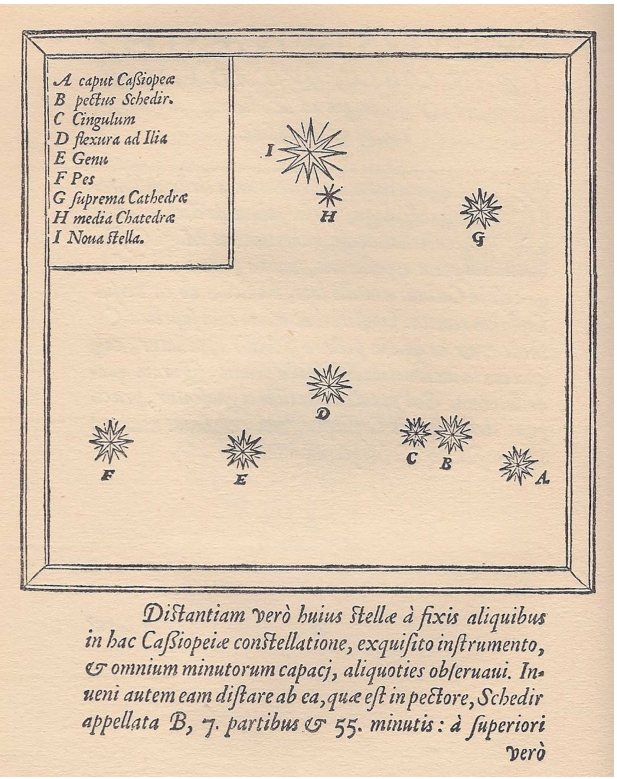

Figure 2. Star map of the constellation Cassiopeia showing the position of the supernova of 1572 (the topmost star, labeled *I*); from Tycho Brahe's *De nova stella* (from Wikipedia. This work is in the public domain in its country of origin and other countries and areas where the copyright term is the author's life plus 100 years or less).

multi-well plates. They demonstrate that use of an automated image cytometer can decrease validation times from ~40 min per 96-well plate to 8 min. This technique may have high utility in speeding up and bringing increased automation to the single cell sorting and cloning process.

Paiè and colleagues (this issue, page 987) review the state of the art for incorporating on-board imaging solutions into lab-on-a-chip platforms. These so-called microscope on chip (MOC) have been developed using a range of technologies including transillumination lens-less MOCs and mobile phone-based MOCs. This review points the way toward developing mass produced miniaturized microscopy solutions that can be deployed in a range of settings such as point-of-care diagnostics and "in the field" scenarios.

In many ways the quantitative measurement of astronomical features in the centuries succeeding Tycho Brahe are not so different from the quantitative measurements made in image cytometry (6) and to some of the approaches and techniques described in the articles in this issue. In both cases, measurements may be made by different instruments, each with varying wavelength ranges and using different contrast mechanisms. In both cases, a common goal is to extract as much meaningful information as is possible regarding the subjects of study. Similarly, both fields of study have highly benefitted from technological advances, especially those in the fields of optics, photonics, and imaging. Finally, due to the diverse nature of the image data and corresponding measurements, standardization of the image data and derived quantitative measurements across all instrumentation platforms is a laudable and complex challenge. However, there will likely be many benefits to developing such a standard, as we have already seen in the flow cytometry field with the development of MIFlowCyt.

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