TRPGR: A field-based high-throughput phenotyping platform for plant genetics

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Scientific Objectives and Intellectual Merit

The essence of plant biology is to understand the link between genetic variation and observed phenotypes. The association of DNA polymorphisms with phenotypes on a genome-wide scale and dissection of the genetic architecture of complex traits on a species-wide level requires accurate phenotyping of large populations along with genome-wide molecular markers. Genotyping can now be accomplished with low-cost, high-throughput assays and sequencing. Phenotyping, on the other hand, is labor intensive and has become the limiting factor in plant biology studies and crop improvement programs. Platforms for high-throughput phenotyping (HTP), particularly in field environments, are needed to compliment the wealth of genomic information in plant genetics. This project will develop transformational capacity for field-based HTP by creating platforms for rapid assessment of multiple quantitative plant traits. Proximal sensing tools and GPS referencing will be integrated to develop mobile platforms that are low-cost, flexible, robust, and amenable for a range of species. A central premise of this work is that integrating streams of data from multiple types of sensors will increase both the accuracy and number of traits that can be quantified for field grown plants. Thus, a second objective is to develop novel algorithms for analyzing HTP data to quantitatively estimate phenotypes expressed in field environments. The newly developed sensor systems, indexes, and algorithms will be applied in test cases that examine multiple quantitative traits in wheat, soybean and cotton.

Broader Impacts

Over the last decade, continuous improvements in genotyping technology, especially sequencing platforms, have radically increased the amount of genotypic data available, while lowering costs per datum. In contrast, phenotyping of large populations for multiple traits in multi-environment field trials remains costly and laborious, especially for dynamic traits that are repeatedly measured during the life cycle of a plant. To truly enable 21st century biology in the genomic and information age, HTP platforms must be developed with the capacity to quantitatively assess thousands to millions of plant phenotypes. Such platforms will compliment the explosion of genomic information currently being collected and will enable field level experimental biology on a scale that has not been previously possible. New platforms can also expand the germplasm evaluations in plant improvement programs and thus directly benefit attempts to address the challenges of increasing food, feed, and fiber production in the face of growing populations, decreasing arable land area and global climate change. This project will focus on field-based assessment of economically important crop species that represent very different plant types and production systems, helping to ensure broad applicability of the platforms and associated analytic methods to a range of target environments and species, and scalability for larger studies. The tools and methods developed will be transferred to the plant genetics and crop improvement communities through hands-on training workshops, student internships, a project website and Internet-based resource for training materials, equipment designs and software. HTP workshops and an international symposium will be hosted to promote technology dissemination nationally and internationally. This project will provide training for graduate and postgraduate scholars while hosting multiple internships for undergraduate engineering and plant science students in the exciting field of HTP.
RELEVANCE AND JUSTIFICATION

Perhaps the greatest challenge of plant science and crop improvement in the 21st century is predicting how a plant’s appearance (phenotype) is dictated by its genetic make-up (genotype). Spectacular advances in “next generation” DNA sequencing are rapidly reducing the costs of genotyping. In contrast, methods for rapid characterization of plant traits (phenotypes) have evolved little over the past 30 years, and accurate measurement of physiological and developmental traits on large sets of germplasm remains problematic. This is especially true for complex traits (i.e., traits with polygenic inheritance and influenced by the environment) that vary over the life history of a crop species. Complex traits are of primary interest to crop improvement because the majority of important agronomic traits are under multigenic control. Among the most challenging and least tractable of such traits are those related to heat and drought stress tolerance.

Recognition of the phenotyping constraint has stimulated development of the field of phenomics or high-throughput phenotyping (HTP), which seeks to accurately characterize large numbers of individuals or populations using a fraction of the time and labor of manual phenotyping methods. In plant science, HTP has largely focused on robotic greenhouse systems using plants grown in pots. While these systems are valuable, their utility for characterizing crop responses relevant to field situations is unclear and the scalability of such systems is limited. Thus, there is an urgent need for robust field-based HTP (FB-HTP) systems for measuring agronomically important traits at field scales and associating them with their genetic determinants. However, implementing FB-HTP requires integration and synchronization of phenotypic observations, instrumentation, infrastructure and analytic tools, potentially including static and ecophysiological modeling (physiological mechanisms underlying ecological observations).

In the realm of phenomics, two major challenges are 1) the measurement of traits on very large genetic populations and 2) the repeated measurement of dynamic traits (i.e., traits that have a phenotype that changes with time). Many new statistical approaches to characterize the underlying genetic basis of complex traits (i.e., genetic architecture) rely on large populations of germplasm. These populations are typically on the order of thousands of distinct lines/individuals. In model plant species with small physical stature, such populations can be evaluated in greenhouse or growth chamber environments. However, for many species, particularly those of agronomic interest, large populations require enormous field experiments. This then becomes the primary limiting factor for dissecting the genetic architecture of complex traits. While mechanized planting facilitates establishing large field trials, the real research limitation comes when phenotypes need to be measured. Measuring a single phenotype by hand might take 15-30 seconds per plot. When applied to trials involving thousands of entries, great investments of time (on the order of days for a single measurement) and resources are needed to measure a single trait (15 seconds across 6000 plots requires three 8+ hour days), and measuring within a narrow diurnal time range (e.g., 1400 to 1500 h) is exceptionally challenging.

These limitations often result in the measurement of traits at a single fixed time point, typically at or near plant maturity. With this approach, it is only possible to estimate the accumulated effects of individual quantitative trait loci (QTL) from the beginning of plant development to the fixed time of observation. Notably, separate QTL could be differentially expressed throughout the developmental program of a trait but have the same or different final effects. Thus, when connecting genotype to phenotype, it is important to consider the expression dynamics of individual QTL throughout the development of the plant and their interaction with the environment. The successive measurement of phenotypic traits throughout plant development is essential for QTL mapping of dynamic traits, but it is laborious and costly when measuring phenotypes manually.

A promising approach for FB-HTP is to adapt techniques from remote sensing for detection and estimation of phenotypic traits. These adapted techniques follow the same physical principals used with satellite and aerial sensing, but because of their close-range deployment, are denoted as “proximal

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\(^a\) The Triticeae Coordinated Agriculture Project (T-CAP) is currently developing Barley and Wheat nested association mapping populations on the order of 10,000 lines for each species.
sensing". Previous studies in characterizing crop responses to water and nutrients, and to a lesser extent for breeding and genetics, have established that numerous plant traits can be measured reliably using proximal sensing\textsuperscript{9,10}. Proximal sensing with non-contact spectral, infrared, or ultrasonic sensors has great potential to speed phenotyping with a coincident reduction in required labor and time, but it requires systems for deploying instruments in a consistent, repeatable manner\textsuperscript{11}. The mounting of non-contact sensors to a high-clearance vehicle system can potentially harness the full capability of proximal sensing in a reliable, flexible, and efficient system that operates at small-plot to plant-level spatial scales. Combining this approach with GPS positioning to spatially locate the proximal sensing data (georeferencing) thus appears capable of delivering a robust FB-HTP platform.

A primary challenge for FB-HTP becomes how to synthesize this information. The data collected through HTP (e.g. light reflectance at 850 nm) are indirect proxies for phenotypes of interest such as leaf chlorophyll content, leaf area index, or yield, so more direct indicators of crop responses are needed. In this project, we will focus on integration of multiple layers of data and employ inverse modeling approaches to precisely describe complex plant phenotypes. We hypothesize that inverse modeling will provide a transformative increase in the amount of meaningful biological data on a level never before demonstrated.

In summary, an effective FB-HTP approach will integrate 1) robust multi-sensor platforms capable of rapid field deployment and assessment and 2) modeling of phenotypic data through the use of regression, plant growth simulations and related techniques. The overall goal is to integrate multiple measurements from proximal-sensing data with plant growth simulations to maximize the biological utility of the estimated phenotypes.

**PROJECT GOALS**
The central hypotheses of this research project is that FB-HTP combined with inverse modeling at multiple temporal scales can be used to accurately assess complex plant phenotypes and will provide a transformative increase in the amount of biologically meaningful phenotypic data.

Following this hypothesis, the objective of this project is to develop novel FB-HTP tools and resources for plant genomics research including algorithms to support FB-HTP analysis. We will focus on evaluation of complex physiological traits such as canopy architecture under water deficits (as affected by leaf size, leaf angle, branching or tiller development and plant height), plant recovery from water deficits (characterized by rates of transpiration and growth), and plant maturation under high temperature stress (evidenced by declining leaf area index and increased canopy temperature). HTP evaluation will be conducted at multiple temporal scales on distinct crop species under field conditions to explore both the utility and potential of FB-HTP. The development of these techniques and associated FB-HTP resources are expected to deliver tools for plant genetics studies that will substantially increase our ability to characterize in detail, huge collections of plant genetic resources.

**Specific Objectives.**
1. Develop FB-HTP platforms consisting of proximal sensors in an open-source framework and state-of-the-art GPS guidance systems mounted on high-clearance field vehicles.
2. Develop efficient and easy-to-use pipelines to process FB-HTP data and disseminate associated algorithms.
3. Develop algorithms to estimate biological traits from FB-HTP data using inverse modeling with statistical and biophysical models. The focus will be on identifying modeling techniques that optimize phenotypic information from raw HTP data.

**EDUCATION AND OUTREACH**
This project will serve to advance the use of FB-HTP in plant genomics research. This will be achieved by 1) integrating summer internships for undergraduate students from multiple disciplines, including engineering and plant science, 2) providing graduate student training and postdoctoral development, 3)
hosting yearly workshops for HTP training and 4) hosting of research findings on a project web-site, 5) hosting of HTP workshops at Plant and Animal Genome conference.

Project team: This project brings together an interdisciplinary team of researchers with the breadth of expertise needed for successful implementation of FB-HTP. PI Poland is an early career scientist focused on wheat genetics with expertise in high-throughput sequence-based genotyping, quantitative genetics and genomic selection and has not had previous projects through the Plant Genome Research Program. Collaborator Fritz is an experienced wheat breeder with extensive knowledge of wheat germplasm and field evaluation. Collaborator Schapaugh is also an experience breeder in soybeans. With similar research focus in cotton, collaborator Gore has experience with association mapping and cotton germplasm. Collaborators P. Andrade-Sanchez, A. French, and R. Price have engineering backgrounds with considerable experience in precision agriculture and remote sensing, a critical component for developing a complete FB-HTP system. CoPI K. Price has an extensive career generating, processing and applying remote sensing data to crop yield predictions. Collaborators J. White and K. Thorp are experts in plant growth modeling and development of data processing pipelines for handling the vast quantity of data that will be obtained from our HTP approach. Collaborator Welch has experience with inverse modeling and with image processing techniques being developed and used in another NSF-PGRP project.

PRELIMINARY RESULTS

HTP: Sensors, Platform, and Vehicle   We converted a high-clearance sprayer into a field vehicle for proximal sensing of crop canopies (Figure 1). By retrofitting a front-mounted boom, we developed a high-clearance vehicle with the functionality to simultaneously measure plant height, canopy spectral reflectance, and canopy temperature in a four-row configuration. Plant height is measured with four sets of Pulsar dB3 ultrasonic transducers and Blackbox 130D level controllers. Canopy light reflectance is actively measured in three bands (670, 720, and 820 nm) with four ACS-470 Crop-Circle sensors. Canopy temperature was measured with a set of eight Apogee infrared thermometers (IRTs). Each scanned row was instrumented with two IRTs, one pointing nadir and the other at 30° from nadir. (The bi-directional combination of IRTs helps separate canopy and soil temperatures.) All data obtained with these sensors were collected with two synchronized data loggers (Campbell Scientific CR3000 and CR1000). The antenna of a Trimble AgGPS-332 RTK receiver was mounted in the center of the boom to accurately locate the sensors. CR-Basic code was written to continuously record analog signals from sensors and serial communication of NMEA-GGA GPS strings at a frequency of one record per second. An algorithm was developed to transform raw data collected from the sonar transducers into plant height measurements. This algorithm was initialized with an elevation survey to define a reference plane (ground level) at planting. After setting the reference plane, raw sensor data were combined with values of sensor-to-GPS antenna to solve for plant height. Canopy temperature data were processed according to their position between rows and their orientation. Canopy spectral reflectance information at 820 nm (near-infrared) and 670 nm (red) were used to calculate the Normalized Difference Vegetation Index (NDVI; Fig. 2)

Field Experiments

In cotton, water availability underpins the incidence of heat stress in the low desert production region of Arizona. A large vapor pressure gradient exists between the leaf and low humidity air, providing a means for leaf cooling by transpiration under well-irrigated conditions. In this “heat avoidance” mechanism, high transpiration rate promotes evaporative cooling of the canopy to temperatures that are markedly lower than ambient air. Under these environmental conditions, there is sustained water loss that can dramatically reduce the relative water content of plants. In response to water deficit, plants decrease

Figure 1. Proximal sensing of cotton
stomatal conductance to minimize transpiration (i.e., water loss). However, reduced transpiration under hot, sunny conditions decreases leaf cooling, which increases canopy temperature and, consequently, the occurrence of physiological heat stress. As a demonstration of FB-HTP, proximal sensing with the high-clearance tractor was used to monitor at multiple times across multiple days the physiological changes of a cotton recombinant inbred line (RIL) population, TM-1 × NM24016, cultivated under well-watered (WW) and water-limited (WL) treatments (Gore et al, unpublished).

We found canopy temperature and NDVI to have significant genetic ($P<0.05$), treatment ($P<0.05$), time ($P<0.0001$), and time-by-treatment effects ($P<0.0001$). Importantly, there was a strong positive correlation ($R^2>0.5$) between week-to-week measurements of both traits, suggesting a highly repeatable phenotypic response of RILs to drought stress. The strongest phenotypic differences for these two traits among RILs within and between water treatments occurred in the early afternoon relative to the two earlier times. Therefore, it was not unexpected that higher broad-sense heritabilities (WW and WL $H^2$: 0.5-0.8) were typically observed for canopy temperature and NDVI in the early afternoon. The canopy temperature of RILs gradually increased for both WW and WL plots from morning to afternoon. However, the average temperature of WL plots reached 37°C or greater in the early afternoon, but the average temperature of WW plots seldom exceeded 33°C. NDVI essentially remained unchanged for WW plots throughout the day, while NDVI gradually decreased from morning to afternoon for WL plots. The lowest NDVI values were recorded for WL plots at 1pm. We hypothesize that the conformational change of leaves from wilting exposed more soil relative to non-wilted plants in WW plots, thus decreasing NDVI (i.e., less greenness) for WL plots.

During the 2012 season, we evaluated multiple panels of winter wheat germplasm using a similarly configured prototype HTP platform. NDVI, plant height and canopy temperature were measured at multiple timepoints over the growing season. HTP measurements were made at 5 Hz resulting in 10-20 measurements per plot depending on ground speed and plot size. Through assigning HTP measurement to experimental entries, we were able to make accurate determination of NDVI and plant height values.
For plant height, we observed significant differences between plots that differed by as little as 4 cm. Likewise, NDVI measurements were very accurate, giving significant differences between plots at levels of 0.02.

These results illustrate the great potential of FB-HTP for the large-scale physiological study of diverse germplasm and emphasize the need to consider diurnal variation in traits. Successful implementation of a FB-HTP system requires that sensing equipment be used to rapidly screen thousands of genotypes in the field at frequent intervals. Our proposed FB-HTP system will be able to directly measure canopy reflectance, height, width, and temperature. Forward and inverse modeling can then be used to extend the FB-HTP dataset to phenotypes beyond what can be measured directly.

BACKGROUND

Estimation of Phenotypes through Inverse Modeling. Development of optimal and efficient FB-HTP algorithms requires adaptable approaches for integrating observational data with underlying biological and physical models. Any given numerical model typically is used to convert attributes of a system into observational data. Inverse modeling does the reverse, converting observational data into inferred system attributes. The latter situation corresponds to the target of our FB-HTP research: we seek to use observed values obtained from proximal sensing to estimate attributes of plant communities, with a specific focus on phenotypes of individual lines or accessions. For our purposes, models per se are usefully divided into two broad categories, statistical models and biophysical models. The first class includes regression and other models where traits are estimated through knowledge of the system, modeled relations emphasize statistical goodness-of-fit. The second class relies on prior knowledge of physical laws and crop growth processes to specify relations. The models may be static, or they may involve dynamic simulation.

Reasons for choosing to use one class of models over another are wide and varied, but most importantly are centered on factors relating to model robustness, ease-of-implemention and uniqueness of results. In our current and proposed research, a range of model types within each class will be tested because there is widespread debate and uncertainty over the optimal approach for estimating phenotypes from proximal sensing datasets.

Statistical Modeling. Standard regression approaches for converting observed spectra and other remotely or proximally acquired data into phenotypic traits are readily available. Examples include LAI from NDVI\textsuperscript{12} and canopy transpiration from micrometeorological data and canopy temperature\textsuperscript{13}. In addition to these approaches, we will use more novel “large p, small n” modeling approaches, such as Partial Least Squares Regression (PLSR)\textsuperscript{14}, ridge-regression (RR)\textsuperscript{15}, and random-forest (RF)\textsuperscript{16} among others, to model full-spectrum data for phenotypic assessment. Many of these approaches have found application in genomic analysis of large genotypic datasets\textsuperscript{17,18}, which are somewhat analogous to the multi-spectral data collected through proximal sensing. Once a statistical relation is modeled between spectra and plant traits, it can be used to translate large sets of multispectral data to observed phenotypes. PLSR is widely used to characterize crop biophysical and biochemical attributes and to predict green biomass, leaf area index, leaf chlorophyll concentration, leaf chlorophyll density, leaf nitrogen concentration, and leaf nitrogen density, quality and yield\textsuperscript{9,10,19-22}, and the basic approaches are applicable across crop species\textsuperscript{22-24} and at different spatial scales. Nonetheless, success varies by crop/vegetation type, and additional work is needed to refine the methods for specific crop types and geographic regions.

Statistical modeling with time-trait components can also give a more accurate picture of plant growth and

![Vegetation Curve and Associated Vegetation Phenology Metrics (VPM)](image)

Figure 3. Vegetation Phenology Metrics (VPM) developed through modeling multiple measurements of NDVI throughout the growing season. (Figure adapted after Reed et al. 1994)
development over a day or the full growing season. An example would be analysis of diurnal canopy temperature to estimate the maximum rate of increase in canopy temperature on a day with severe stress. On a seasonal scale, vegetation indices derived from repeated field measurements can be used to create profiles of crop growth and development. One approach for using repeated measurement profiles is to derive Vegetation Phenology Metrics (VPMs). Metrics that can be derived this approach include the onset of greenness (crop emergence), rate of crop growth (rate of green-up), time of maximum greenness (flowering date for some crops), rate of senescence of the crop, end of greenness (full senescence or end of the growing period for that crop), length of greenness, accumulated NDVI (area under the curve) (Figure 3). This approach can be used to describe a range of plant phenology and seasonal growth characteristics, including yield. The underlying appeal of the VPM concept lies not in the strength of the vegetation index, or the resolutions of the proximal sensing systems, but rather in the high temporal frequency of the spectral measurements during the plant grow period. This type of frequent measurements can be made tractable through FB-HTP.

**Biophysical Modeling.** Biophysical models are constrained by knowledge incorporated from plant science, ecology, physics and other disciplines. These models, therefore, are thought capable of predicting plant traits more robustly and with greater detail than statistical models. Thus, essential to our envisaged inverse modeling approach is the availability of models that incorporate equations of physical or biological processes to represent a plant ecology or cropping system of interest. Software is then implemented using available observations to estimate unknown model parameters, including phenotypes of interest. Since the model is based on physical or biological equations, parameter estimates from inverse modeling have physical or biological meaning, allowing greater generality, applicability and robustness. Hence, our approach should have minimal reliance upon locally calibrated statistical models, where estimated model coefficients lack direct physical or biological meaning and extension to other geographic locations or growing conditions. As an example, a plant growth model might predict plant biomass and leaf area index (LAI) given parameter estimates for root-shoot partitioning (P1), maximum radiation use efficiency (P2), reference single leaf area (P3), and other inputs (Figure 4). In a “forward modeling” scenario, the parameters P1, P2, and P3 are known for a given cultivar or line, and the model is run one time to simulate the biomass and LAI response. With inverse modeling, these model parameters are estimated from the measured responses of LAI or biomass. In practice, inverse modeling often requires an optimization algorithm, which runs the model forward many times, adjusting the input parameters to achieve some objective, such as minimizing the error between observed and simulated LAI. Thus, for our hypothetical case and considering a single cultivar or line, traits represented by parameters P1, P2, and P3 would be estimated using inverse modeling of the time series of field measurements for canopy reflectance, height, width or temperature. Since the estimated parameters are underlying (unobserved) plant traits, they derive from more fundamental plant processes than values for plant height or canopy reflectance measured at a single time, are constrained by physiological information embodied in the model, and are obtained from the synthesis of a large number of observations, their estimates should have less error than the direct measurements of canopy height or reflectance and thus have higher heritability.
and stronger associations with genetic markers.

Among the simplest biophysical models are those used in functional growth analysis, where time series growth data are fitted to curves. An intermediate level of model complexity is found in static canopy reflectance models such as PROSAIL. At a highly mechanistic level, dynamic simulation models explicitly incorporate ecophysiological principles. Many parameter values are specified based on prior knowledge, which helps limit the parameters to be estimated through inverse modeling. Tardieu and Tuberosa provide an example of using inverse modeling for estimating traits related to abiotic stress tolerance in plants. Other examples of inverse modeling in agriculture are found especially in research of hydrologic and nutrient processes at field and watershed scales.

While potentially transformative in its ability to estimate large numbers of plant traits from FB-HTP data, inverse modeling presents challenges and risks. A detailed mechanistic model may embody more principles of ecophysiology than a simpler model, but increasing the number of parameters can decrease model accuracy and increase computation time. A related concern is that due to incomplete knowledge of crop growth processes, simplifications in model design, and input parameter error, different models or configurations of a single model may provide results that appear equally reasonable, a condition known as equifinality. The fundamental question in dealing with more complex models is whether we can constrain model inversion sufficiently to obtain meaningful estimates of phenotypic traits.

PROJECT PLAN
This project will develop, evaluate and deploy a robust FB-HTP platform that will greatly accelerate the genetic dissection of complex traits among genetically diverse plant populations and provide the foundation for adapting FB-HTP platforms to a range of plant species. Our approach emphasizes integration and innovative analysis of multiple data streams obtained through proximal sensing. Our specific aims are to:

1. Develop a flexible open-source field-based HTP platform. We will develop a flexible open-source HTP data collection platform based on a high-clearance vehicle. The main function of this platform is for...
sensor deployment to achieve rapid measurements of spectral reflectance, infrared thermometry, and height measurement with geospatial data referencing of all sensor output.

**HTP platform design**

**Vehicle frame and power** - We will use high-clearance (1.0-2.0 m) four-wheel drive vehicles to pass over plants/plots without disturbance and to ensure field processing under wet conditions. Use of hydraulic drive will facilitate precision operation under variable speeds. The platform will provide surge-protected 20 A at 12V DC power for instrument operation and support sensors on two tool-bars extending from the front and back axles, both adjustable with hydraulic cylinders. Multiple pivot- points will allow vertical movement of the sensor-bar frame while maintaining a horizontal orientation. The arm dimensions will allow adjustments ranging between 0.25 and 2.5 m above the ground.

**Real Time Kinematic (RTK) Global Positioning System (GPS)** – We will employ RTK-GPS for georeferencing incoming data and for vehicle guidance. By integrating RTK-GPS receivers with the proximal sensing equipment, we can determine sensor position with cm-level accuracy. This approach will enable continuous measurements with sensing equipment and parsing of collected data to experimental plots (also georeferenced). Existing tractor auto-steering technology will be implemented to achieve cm-level driving accuracies throughout a growing season. With RTK-GPS, we can ensure consistent platform position and speed during multiple field deployments.

**Data acquisition system.** We will employ ruggedized data loggers designed to withstand vehicle vibration, high temperatures (40+ °C), dust and high humidity. Loggers will provide 50 differential-voltage channels for analog measurements, multiple serial ports for serial communications, and external memory devices for data back-up. We will also implement web-based and radio wireless communication protocols to enable real-time monitoring and quality control of input data.

**Open-source engineering.** To ensure that other research groups can adapt our approach to specific crops, locations and equipment, we will emphasize use of instruments where the sensor outputs are fully documented and accessible with third party data-logging hardware. Similarly, software developed for the platform will be open-source and engineered for ease of re-use and modification.

**Field deployment of HTP platform** - As noted in the design features for this project, the HTP platforms developed here will collect measurements in close proximity to the plants while in motion. This ground system will be front-mounted with a suite of sensors that include:

**Radiometric infrared thermometers (IRT).** We will use commercially available IRTs to measure canopy temperature (IRT model SI-121 narrow field of view -18 degree half-angle manufactured by Apogee Instruments, Logan, USA). Each IRT will be individually calibrated using an Advanced Kinetics extended aperture blackbody in a constant temperature room (accurate to <0.1 K).

**Active spectral sensors.** We will use active-light sensors to measure canopy reflectance. The use of active-light sensors will enable more robust data collection in varying light conditions as needed for FB-HTP. We will initially use active-light sensors with user-selectable filters and a combination of 670, 720, and 820nm filters (ACS-470 manufactured by Holland Scientific, Lincoln, NE).

**Displacement sensors (SONAR).** SONAR will be used to quantify plant height in one dimension. We will use pulsed ultrasonic transceivers of 125 kHz with a 12-degree beam angle. In combination with the vertical reference from the RTK-GPS (±2 cm) the distance to the ground and the top of the canopy can be determined with the difference being plant height.

A key component of this system will be the integration of GPS coordinates with all proximal sensing data collected through the various sensors. Measurements will be individually georeferenced with simultaneous recording of position from the NMEA-GGA GGA (National Marine Electronic Association) string output by a GPS-RTK receiver with GLONASS reception enabled. We will develop new approaches to maximize the rate of data recording to increase resolution of measurement, add precision to georeferencing positions, and increase the number of measurements within each field plot. Measurements will be scheduled throughout the growing season to record the dynamics of plant growth, and repeated at different times of the day to capture changes during the diurnal cycle.
**Future developments in FB-HTP platform technology.** The open-source field-based HTP platform will be continuously upgraded as we gain knowledge on optimal configurations for the mechanical and electronic systems and their interface with the crop plants and ambient conditions. We will present all equipment specifications and configurations through our project website and prepare manuscripts to ensure open access to these new approaches. Our workshops will also be used to display the equipment, provide training to others in the HTP community and encourage comparisons of promising instruments.

We will explore the use of full-spectral data collection (spectroradiometers) and image collection on the FB-HTP platforms. To date, analyses of plant-scale images in HTP have mainly relied on stationary platforms with mounted camera. This approach requires moving or placing plants in an imaging chamber and is primarily suitable for greenhouse or growth chamber studies. To develop HTP imaging for field-based experiments entails evaluating different mounting and system setups on the HTP platform. Likewise, spectroradiometers can capture a much fuller picture of the plant canopy than measurements at a limited number of wavelengths as produced by the active spectral sensors. We will evaluate configurations and solutions for spectroradiometer and image data collection on the HTP platform.

Field deployments of this platform will enable us to explore optimal configurations of field plot layouts for HTP data collection. We will be able to make recommendations on various aspects of field plot layout, including row length and number of rows (plot width), to increase the effective capture of HTP data. From a perspective of future developments, the successful implementation of this platform in HTP projects will foster the design of reactive mechanical systems with automatic boom-height control for precise measurements from a uniform distance, implementing a side motion to a sensor suite to reduce the number of sensors, implementation of auto steering and autonomous data collection, and other innovative approaches to FB-HTP.

**Evaluation of HTP platforms on field-trials of wheat, cotton and soybean.** HTP platforms will be evaluated in field-based trials representative of target production environments. The performance of the platforms and the algorithms will be assessed in each field trial through: 1) direct comparison FB-HTP traits with conventional field measurements of the same or related traits (‘ground truthing’), and 2) comparison of repeatability/precision for measurement of individual traits vs. rapidity or cost of assessment. Field trials are routinely planted at each of our research locations in Arizona and Kansas. Each location is equipped with meteorological stations to provide accurate weather data needed for plant growth modeling.

**Phenotypic Evaluation of Cotton.** We will evaluate two recombinant inbred line populations that exhibit significant genetic variation for physiological and agronomic traits that are important for adaptation of cotton to hot, arid environments. Both populations are in the process of being marker saturated through genotyping-by-sequencing. The first population of 95 recombinant inbred lines (RILs) is a cross between the stable, interspecific inbred line NM24016 \( (G. \text{hirsutum}/G. \text{barbadense}) \) and elite inbred line TM-1 \( (G. \text{hirsutum}) \). A second population of 170 RILs from a cross of Pima cultivars PS-6 \( (G. \text{barbadense}) \) and 89590 \( (G. \text{barbadense}) \), their parents and four commercial check varieties will be evaluated concurrently in replicated tests at Maricopa. Tests at Maricopa will include two replicates each year, and the tests will be planted as augmented incomplete block designs using parental lines as repeated checks. The two tests will be conducted under two irrigation regimes (well watered and water limited). The start of the water deficit will coincide with the average first flower date of the RIL population, which is typically the first week of July. This minimizes the interaction of flowering time and water deficit, which can be a confounding factor when evaluating stress tolerance.

Measurements of canopy temperature, plant height and vegetation indices will be collected weekly at multiple times using the tractor-based platform throughout July and August, which coincides with the critical stage of peak flowering and boll development as well as the peak seasonal temperatures in central Arizona. Other visual and laboratory measured agronomic and physiological traits will include time to

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\(^{b}\) M. Gore et al, unpublished
Phenotypic Evaluation of Winter Wheat. For wheat (*Triticum aestivum*), we will evaluate elite breeding lines and an association mapping population in field trials in production environments in Kansas. Trials will be planted at Manhattan, KS in 6-row (1.2m) x 3.6m plots in an augmented incomplete design with two reps. Trials will be evaluated for grain yield and quality. Simple physiological and morphological traits including plant height, flag leaf length and width, as well as disease severity and heat tolerance (green leaf duration) will also be assessed.

We will utilize a panel consisting of 300 hard winter wheat cultivars and elite breeding lines. This panel has a range of diversity and maturity and significant genetic variation for all traits of interest. The panel has been genotyped with a 9K wheat SNP array and genotyping-by-sequencing (GBS) (E. Akhunov, J. Poland, unpublished - to be publically available by mid-project). Collection of precise FB-HTP data on this panel will enable future analysis via association genetics.

We will focus testing on 250 elite inbred lines from the KSU wheat breeding program that enter yield testing each year. These lines are candidates for release as varieties and will have good general adaptation across the state. The trial will be planted in augmented incomplete block designs at six locations, two of which will be targeted for HTP. We will take hand measurements of plant height, flag leaf length and width and heading date. We will also visually rate disease severity for common diseases including leaf and stripe rust. These plots will be harvested and grain yield measured.

We will use the HTP platform to take measurements of canopy temperature, plant height, and vegetation indices (NDVI) at 7 day intervals from early March through late May corresponding to early green-up through flowering and grain-filling for winter wheat in Kansas. We will take limited measurements (1 or 2) after fall planting to evaluate emergence and to provide baseline elevation data for all fields. Green-leaf duration (stay-green) is a key trait for performance under heat and drought stress in Kansas. With multiple repeated measurements of NDVI, we will be able to perform several time-series and multivariate analysis of leaf duration and the genetic bases of this trait along with the effect on agronomic performance. We will take visual evaluations of foliar leaf diseases (primarily leaf rust) and flowering date. We will take hand measurements of plant height, flag-leaf length and width, specific leaf area, leaf thickness, and chlorophyll content. We will measure yield from mechanically harvested plots and grain composition including 1000 kernel weight, test weight and protein.

Phenotypic Evaluation of Soybean. To evaluate FB-HTP in soybean, we will focus on a set of “historical” cultivars including 60 maturity group III (MGIII) and 54 maturity group IV (MGIV), soybeans released from the 1920’s until 2010. Genotypes will be grown in a complete randomized block design with four replications per genotype. Genotypes will be planted in 4-row plots, 3.4m long, and spaced 76cm apart. Evaluations were completed in irrigated (flood irrigation) and dryland environments. Full-spectral data will be collected using an ASD FieldSpec 3 spectroradiometer (Analytical Spectral Devices, Inc, Boulder, CO) on near cloud-free days within 2 hours of solar noon with optimization and white reference calibrations (Spectralon Labsphere Inc.) at 7-10 day intervals during seed set. We will concurrently use the FB-HTP vehicle platform to take measurements on plant height, NDVI, and canopy temperature. Measurements on plant height and flowering date will be taken by hand. Plots will be mechanically harvested and measurements on grain yield and seed quality will be taken.

2. Develop efficient and easy-to-use pipelines to process FB-HTP data and disseminate associated algorithms

The overall objectives of developing a data management pipeline for HTP are 1) data management and access within this project, 2) promotion of community-based standards for managing and analyzing large sets of FB-HTP data and 3) interaction with community databases to integrate tools and schema for utilization of HTP in plant genomics research.
The planned proximal sensing and modeling activities require integrating, managing and processing large datasets from diverse sources. We propose to develop a data management pipeline (DMP) that builds on existing standards and emphasizes open source software. The envisaged DMP will be designed for final deployment in the iPlant Discovery Environment\textsuperscript{48}, (www.iplantcollaborative.org/discover/discovery-environment) or a similar platform. We will interface closely with community databases to ensure that HTP data standards will integrate with established data structures currently in use. For initial development and testing, the DMP will rely on a relational database hosted on the project server that merges elements of the ICASA standards and ICIS. Once the required database architecture is established, the option of using “intelligent files,” where the contents are largely self-documenting, will be considered. Such files can allow data processing with a wide range of software without reformating, and they may avoid inefficiencies that arise in mapping the diverse data onto a relational architecture. The NetCDF format\textsuperscript{49} is widely used in remote sensing and is of interest for arrays. Discipline-specific XML formats will be evaluated, including the Ecological Metadata Language (EML; knb.ecoinformatics.org/software/eml) and the Geography Markup Language (GML; www.opengeospatial.org/standards/gml). Preference will go to adapting existing standards to minimize proliferation of standards \textsuperscript{50}.

HTP data will have several layers of processing prior to being in a form for genetic analysis. To conduct physiological modeling primary data must be linked to environmental and experimental conditions. For a given experiment, key information includes field management and environmental data, the experimental design and field plot designations, sensor and GPS outputs, and complementary phenotypic data such as crop phenology and yield. We will utilize ICASA standards for describing field experiments\textsuperscript{51}, along with standards from Data Management System (DMS) of the International Crop Information System (ICIS)\textsuperscript{52}, Plant Ontology (www.plantontology.org) and CropOntology\textsuperscript{53} for documenting HTP data.

The first step in processing HTP data is to link data from sensors to GPS coordinates for each respective data point. Depending on how the sensor data was recorded on the data loggers, senor data will need to be joined with GPS coordinates. As sensors are arrayed on the tool bar in different positions from the GPS receiver(s), a correction factor based on the sensor architecture must be introduced. Georeferenced sensor data must then be assigned to experimental plots. Ground-based surveying or algorithmic determination of boundaries will define plots. With coordinates defined for plot boundaries, all georeferenced data inside a given plot will be assigned to that experimental entry. We will develop these preprocessing algorithms into a user-friendly software package.

Following assignment of data to experimental entries, calculation of indices and forward and inverse modeling will be done with GIS packages, the R statistical package, or newly developed algorithms. Inverse modeling with ecophysiological models will rely on nonlinear parameter estimation and optimization packages such as PEST (Dougherty, 2010). The model-independent nature of PEST would allow multiple models to be optimized using a single, preexisting optimization program. The comparisons among the various estimates of phenotypes requires that phenotypic data be organized as arrays where all phenotypic data are indexed by plot and genotype identifiers. The software used in joint analysis of molecular and phenotypic data typically require data in a standardized flat file format. We will develop database and other structured digital formats that can readily be exported in formats useful for genetic analysis. We will document the wide range of protocols and methodologies used in the project, using available protocol databases (e.g., prometheuswiki.publish.csiro.au). The main need likely will be to either automate or simplify protocol data entry to assist end-users in document their data acquisition and processing.

The DMP will be developed in four stages, starting with an initial needs assessment and culminating with the finalized DMP. Two formal cycles of testing and revision will be conducted using data from each year of the Arizona and Kansas field trials. Draft databases or structures and software will be distributed via the project web site. In the concluding phase of the project, we will determine the optimal
deployment strategy with options of software tools as standalone programs for use on individual computers/servers or as web-based services (e.g. via the iPlant Discovery Environment).

The initial database will be designed based on our experiences with prototype HTP testing. We have generated data on both cotton and wheat and will use a relational database as the foundation. The architecture will merge aspects of the ICASA standards, the ICIS DMS (www.icis.cgiar.org) and protocol management systems. The relational database will ensure safe storage of data, facilitate data export to specific software tools, and allow examining issues related to processing of large data items such as plot-scale imagery. Initial database access will be through server-based SQL queries, and the main data processing will involve Python and R scripts.

Data processing activities envisaged for the DMP involve 1) initial geo-referencing of the sensor data, 2) uploading data to the database, 3) extracting subsets of data for further processing, and 4) data quality control. The expected processing will follow the activities outlined in the previous section and include calculation of simple traits, simple inverse modeling, inverse modeling applied to ecophysiological models. We expect that due to the large computational requirements, model optimizations will be run on dedicated servers and/or deployed via iPlant cyberinfrastructure. Initial development will largely consist of a relational database, scripts to upload or extract data, and scripts to control external processing. Development will follow a “daily build and smoke test” approach. This involves very frequent testing to ensure that all component software and databases retain interoperability and is recommended where component integration is a concern. Major milestones for releases will be identified through feedback from team members and from potential users, especially participants in the workshops.

All field data will require georeferencing and in much of the analyses, positional information will be of interest. The project will develop geo-processing tools for FB-HTP data within the open-source desktop Quantum GIS (www.qgis.org), which can be run on multiple computer platforms and provides a free and flexible platform for development of software tools that could be freely distributed among groups involved in FB-HTP. We can extend Quantum GIS for FB-HTP data geoprocessing using its plug-in system. We will develop an FB-HTP Quantum GIS plug-in, which will be a set of Python scripts and PyQt graphics user interfaces that apply the native Quantum GIS geo-processing functions to FB-HTP data. The GIS will also support visualization and quality control tools. The R raster package and R tools for visualizing and analyzing multivariate data, notably with the Lattice implementation of Trellis graphics will provide additional options for visualization and quality control.

3. Develop algorithms to infer biological traits from HTP data. Our central hypothesis is that FB-HTP data combined with inverse modeling can deliver a transformative increase in useful phenotypic data. This requires assessing different levels of model complexity and mechanistic detail by estimating plant traits with various models, and then comparing those estimates, as well as results obtained through alternative methods. We will conduct inverse modeling and use the model parameters (phenotypes of interest) and estimates of phenotypes that cannot be directly observed. The comparisons will focus on the broad-sense heritability of the traits (error on a line-mean basis). The most robust phenotypic modeling approaches are expected to generate higher repeatability and heritability. Computational tractability and robustness across species and environments will also be considered.

**Traits obtained directly from conventional field measurements or proximal sensing**

1) *Traits obtained from conventional field measurements.* As a complement to proximal sensing, a large set of traits will be measured through visual observation (e.g., flowering date, leaf wilting scores) or conventional field techniques (e.g., grain yield, leaf chlorophyll concentration). As far as possible, these will be acquired using assistive tools such as bar-coding and voice-to-digital conversion.

2) *Traits obtained directly from proximal sensing.* Many simple traits can be obtained directly as outputs from the instruments. We will measure canopy height with acoustic sensors, canopy temperature with

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infrared thermometry at specific times and dates (e.g., 1400 h on day 53), and spectral reflectance with radiometers filtered at specific wavelengths. These trait values will be directly assessed as phenotypes as described below.

**Traits obtained from statistical models**

3) **Statistical modeling based on full-spectral data.** During the first growing season, we will collect spectroradiometer readings across the row-column blocking design of our experimental fields. Readings will be taken using an Analytical Spectral Device, Inc. FieldSpec 4 spectroradiometer (350 – 2,500 nm, 2,151 bands, 10 nm band widths) and transformed to values of reflectance using a calibrated Spectrolon© white-reference panel. Crop canopy biophysical and biochemical traits will be measured within the field over the growing season. We will use these observed phenotypes with full-spectral data to develop predictive models. Using Thermo Scientific GRAMS AI Spectroscopy software, we will first test least-squares regression to identify optimal band combinations for determining canopy bio-physical/chemical characteristics that are influenced by crop phenotypes. We will also employ ridge-regression, random forest and other algorithms to model full-spectral data to traits of interest using R. Model robustness will be tested using a k-fold cross validation (Jackknife or Bootstrapping) approach. With information on optimal band combinations for modeling specific phenotypes in hand, we can guide development of customized radiometers (fewer bands than a spectroradiometer) for future deployment on the HTP platform.

4) **Traits estimated based on published or readily estimated relations with instrument outputs.** We will use a Vegetation Phenology Metric Approach (VPM) to model repeated spectral measurement of vegetation over the growing season and assess the additional value of the multiple phenotypic measurements made tractable through HTP. We will also focus on diurnal modeling to evaluate rapid changes, particularly in canopy temperature, that occur during a single day. VPM will also be combined with multivariate regression to determine the most robust approach for maximizing utility from the high-temporal data afforded by FB-HTP.

**Traits obtained from biophysical models**

5) **Functional growth curve analysis**. Indices calculated from canopy reflectance will be used to estimate crop canopy growth curves using both calendar and physiological time (growing degree days, GGD). Analysis of these growth curves will provide estimates for several important traits, including canopy growth rate, leaf area duration, days or GGD from emergence to flowering, and days or GDD from emergence to maturity.

6) **Simple canopy model for plant stem elongation and leaf area growth.** Given meteorological data and parameters for radiation use efficiency, water stress, standard leaf size, and physiological time between leaf appearances, the model will simulate biomass and LAI. We will implement inverse modeling to estimate the model parameters based on observed FB-HTP data.

7) **CUPID or SHAW model – Canopy architecture and water balance**. These models can simulate canopy transpiration and microclimate over intervals of a few days based on a description of canopy architecture, root distribution, leaf photosynthetic capacity, soil properties and initial soil water status. By inverting the model using observed canopy temperature data, we will estimate plant characteristics related to stomatal conductance and root function.

8) **Ecophysiological models to estimate multiple traits**. A complete ecophysiological model uses meteorological, weather, management, and crop cultivar information to simulate crop development and crop growth, as well as the effects of water and nitrogen stress on crop growth processes. Assimilated carbon is partitioned to various plant components, including leaves, stems, roots, and grain. We will focus efforts on the Cropping System Model (CSM) as distributed with the Decision Support System for Agrotechnology Transfer (DSSAT). Model inversion will be used to estimate crop cultivar parameters, including reference leaf size, leaf appearance rate, and radiation use efficiency.

We will use a comprehensive range of standard to assess the accuracy of the modeling approaches. We expect that the most accurate ecophysiological plant growth models (in terms of describing the
underlying genetic and biochemical processes) will give the most robust correlation to the observed phenotypes.

Repeatability is an important measure for determining the utility of vehicle-mounted proximal sensing. The intra-class correlation between repeated measurements of a trait on the same experimental entry is known as the repeatability (r) of the phenotype. The repeatability is a function of the variation between plots across the field and technical error associated with measuring the trait. The repeatability of these proximal sensing methods directly influences the acceptable number of tractor-based sensor scans for each species, population, and experiment. Increasing the number of independent measurements on the same entry reduces the variance that technical error contributes and represents the gain in accuracy\textsuperscript{60}. We will evaluate the repeatability of proximal sensing methods by comparing 1) single measurements taken by hand and multiple-measurements take by the HTP platform and 2) back-to-back scanning of experimental plots over a continual time series. The gain in accuracy will be determined by a reduction in variance, providing support for increased repeatability through HTP platforms and informing the number of needed technical replicates at each data collection time point.

Heritability measures of how much phenotypic variation among individuals within a population is due to heritable genetic effects rather than to the environment or to measurement error. Estimates of heritability not only provide evidence for the importance of genetic effects, but they are also useful for predicting genetic gain from selection within the context of an identical experimental design\textsuperscript{61}. In general, traits with high heritability will respond rapidly to selection. We will estimate heritability on a line mean basis within each family\textsuperscript{61} (broad-sense) with variance components estimated from a random effects model analysis across environments per Hung et al.\textsuperscript{62} in ASReml version 3.0\textsuperscript{63}. We will use these estimates to further rank proximal sensing methods based on their expected value in genetic analyses. Relative to traditional measures, HTP trait estimates that give higher heritability will indicate more precise phenotypic measurement.

**PLAN TO INTEGRATE RESEARCH AND EDUCATION**

Provide professionals, postdoctoral associates, and graduate students training in the emerging science of high-throughput phenotyping.

*Workshops and meetings*. Under the project, MAC and ALARC researchers will host two workshops in April 2014 and 2105 for training in FB-HTP principles and practices and for allowing researchers to test and demonstrate novel instruments or vehicles under field conditions. We will also host workshops at the 2014 and 2015 Plant and Animal Genome (PAG) conference help in San Diego during January each year.

Maricopa, AZ is an ideal location for US researchers interested in FB-HTP. The dry, mild winters allow us to conduct experiments outside of the usual summer cropping season, and these experiments are reliably managed for different levels of water and nitrogen deficits. The scarcity of cloudy days also helps to ensure optimal conditions for field observations and evaluation of instruments that require stable illumination can be used during the workshop.

Each year we will hold a four-day workshop at Maricopa to provide opportunities for participants to learn about field instruments, vehicle configurations, georeferencing, data management and inverse modeling while having hands-on demonstrations and field exercises. The first three days will involve daily rounds of lectures, field exercises with vehicles, and data processing. The last day, teams of participants will present results and have an “open-lab” session where they can pursue specific interests or concerns with instructors.

All training material will be prepared as e-learning content to ensure broader distribution and allow updating. Following the conclusion of this project we envisage the workshops becoming annual events that combine training with tool/sensor comparisons.

We will broadly advertise this workshop through the project website, community e-mail list server and websites (e.g. GrainGenes, MaizeGDB) and during presentations (made by PIs and
Collaborators) at international conferences. We look to attract scientist and students who are interested in HTP or starting projects using HTP. To ensure the best available opportunities for hands-on activities at the workshop, the participants will be limited to 30. This project will provide all of the training material and on-site accommodations. Participants will be responsible for travel and lodging during the workshop. To attract a larger representation and give opportunities to individuals who might not be able to attend otherwise, we will offer partial- or full-stipend support for the workshop. Through an application process, we will identify individuals who have strong interest in HTP, a good scientific background in plant genetics and/or biological engineering, and demonstrated need for workshop support. We will target applications from graduate students, post-doctoral scientist and early-career faculty. Applications will be encouraged from under-represented minorities in science and engineering.

In conjunction with the annual PAG meetings, we will host a workshop on HTP applications for plant genetics research. This workshop will focus on 1) symposium type presentation of new advances in FB-HTP and 2) hands-on exercises of HTP data management. As PAG brings leading scientist from around the world, we will have opportunity to invite presentations from scientist who are working on different aspects of HTP. We will organize the symposium side of this workshop to focus on 1) novel advancements in HTP and 2) data-management challenges and solutions in HTP. For the hands-on exercises we will give tutorials on HTP data management and analysis using database structures and software developed in this project.

**Internships.** We will host undergraduate summer interns, targeting under-represented minorities in science, for training in HTP and plant breeding activities. Undergraduate summer internships will be offered each year during the course of this project. Four interns will be hired each summer. One engineering intern and one plant science intern will work at each location (Manhattan, KS and Maricopa, AZ) each year.

One critical and exciting aspect of this research is the collaboration between engineering and plant breeding. It is our intent to bring students from these disciplines together to develop innovative solutions to practical problems. Each student will be mentored by a local PI in their discipline but will be engaged in the overall scope of the project. Engineering students will be involved in calibration and deployment of the FB-HTP platforms and gain experience in remote sensing and electronics engineering while being exposed to plant biology. Plant science students will be trained in phenotyping, small plot research and data analysis, but will also gain knowledge and appreciation for the value of an integrated systems approach. This interdisciplinary approach will foster a vibrant learning environment and prepare students to interface with individuals in complementary disciplines.

**Recruitment plan.** We will broadly advertise the internships by sending announcements to engineering, agricultural engineering and life sciences programs at Kansas State University, the University of Kansas, Wichita State University, the University of Arizona and Arizona State University. In addition, we will work with the Diversity Programs Office in the College of Agriculture and K-State Research and Extension and the Diversity Program with the College of Agriculture and Life Sciences at the University of Arizona to recruit under-represented minorities in science.

**FB-HTP consortium.** The project also will foster establish of an FB-HTP consortium dedicated to promoting open-source development of tools for phenotyping. The consortium would host an FB-HTP web site that contains training materials as text, e-books, screencasts and videos. Topics will include 1) vehicle design and assessment information, 2) instrument design and use, including calibration and maintenance, 3) software tools, 4) datasets, 5) test data for assessing new tools and algorithms, 6) completed datasets to allow reanalysis.
References:


